Cardiac transcriptome study of the effect of heat stress in yellow-feather broilers

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ABSTRACT

The yellow-feather broiler is a popular poultry breed in Asia, particularly in China. In this study, we performed RNA-seq analysis to identify differentially expressed genes (DEGs) in the heart of yellow-feather broilers that had been subjected to heat stress treatment (38 ± 1°C for 8 h, over 7 d) and determine the response of the heart to high temperature and its effects on yellow-feather broiler physiology. We found that body weight (BW) of the heat stress treatment group (BW28 = 354.8 ± 34.8 g) was significantly decreased (p = .033) compared with that of the control group (BW28 = 384.8 ± 58.9 g). However, there was no significant reduction in the heart relative weight (HRW) (p = .538). A total of 37 DEGs related to energy metabolism responded to heat stress in the heart of yellow-feather broiler. The results of KEGG pathways analysis indicated that these genes are involved in oxidative phosphorylation (KO: 00190), cardiac muscle contraction (KO: 04260) and carbon metabolism (KO: 01200). Analysis of the cardiac transcriptome of yellow-feather broilers subjected to heat stress indicated that the heart of these birds has specific physiological mechanisms for regulating body growth in response to high-temperature environments.

HIGHLIGHTS

- Yellow-feather broilers, a popular poultry breed in Asia, were used to determine how the heart responds to heat stress.
- A total of 37 genes in the heart of yellow-feather broilers showed differential expression in response to heat stress.
- The differentially expressed genes (DEGs) are associated with oxidative phosphorylation, cardiac muscle contraction and carbon metabolism pathways.

Introduction

Global warming is affecting the broiler chicken industry worldwide (Windhorst 2006). In the past few decades, broiler production performance, particularly in terms of growth rates, has been significantly improved through genetic selection (Mckay et al. 2000; Deeb and Cahaner 2002). However, high environmental temperatures have a more detrimental effect on fast-growing broilers than on slow-growing broilers (Cahaner and Leenstra 1992; Lu et al. 2007; Cheng et al. 2018), particularly with regards to the body weight (BW) of these chickens (Sohail et al. 2012). Therefore, to enhance performance efficiency, it is important to gain an understanding of the genetic basis of the response to heat stress with respect to poultry physiology.

The yellow-feather broiler is a popular poultry breed in Asia, particularly in China, that is known for its slower growth than commercial broilers and unique meat flavour. Previous studies on chickens have shown that fast-growing broilers develop symptoms of injury to the left ventricle (Olkowski 2007), and failure of the left ventricle can cause loss of blood pumping function (Baghbanzadeh and Decuypere 2008). Birds have an efficient cardiovascular system that delivers the metabolic requirements of nutrients and heat. However, to date, there has been limited research on the response of yellow-feather broilers to heat stress. In this study, we investigated the expression of genes in the left ventricle of yellow-feather broilers with regards to the response to heat stress and analysed the effects of heat stress on the metabolism of these birds.
Materials and methods

Experimental design and animal management

We selected 17-day-old male yellow-feather broilers and raised them in two environmentally controlled rooms, each of which was divided into four cells per replicate. Each cell contained six male yellow-feather broilers, which were acclimated for 5 d. When these birds were 22 d old, those allotted to the heat stress treatment were subjected to a temperature of 38 ± 1°C for 8 h/d over a period of 7 d and maintained at 25 ± 1°C for the remainder of the time. Throughout this time, control birds were housed in a thermoneutral room maintained at 25 ± 1°C. During the experiment, we provided the birds with ad libitum access to feed and water. All the experimental birds were cared for and treated according to the guidelines provided by the Guangdong Ocean University Animal Care and Use Committee (permit number: SYXK 2014-0053).

Phenotypic measurements and sample collection

A digital scale was used to determine the body weight (BW) of each bird at 21 and 28 d of age, and differences in the BW among groups were analysed with a two-way analysis of variance (ANOVA) and post hoc multiple comparisons using SPSS software version 19.0 (SPSS, Chicago, IL). We also determined the heart absolute weight (HAW) of each 28-day-old broiler and calculated the heart relative weight (HRW) as the ratio of HAW to BW (HRW = HAW/BW × 100%). In poultry, relative organ weights are typically assessed as indicators of physiological development (Ven et al. 2013). One 28-day-old yellow-feather broiler from each cell was euthanised at the midpoint of the final heat stress treatment and eight heart tissue samples (four thermoneutral and four heat stress treatment) were collected and stored at −80°C.

Transcriptome sequencing analysis of gene expression in response to heat stress

We used the Illumina HiSeq 2500 platform (Illumina, San Diego, CA) to identify differentially expressed genes (DEGs) in the heart of yellow-feather broilers that had been subjected to heat stress. TRIzol Reagent (Invitrogen, Carlsbad, CA) was used to extract the total RNA from heart tissue samples from each yellow-feather broiler for sequencing, according to the manufacturer’s instructions. A NEBNext Ultra™ RNA Library Prep Kit for Illumina® (NEB, U.S.A) was used to prepare the sequencing libraries according to the manufacturer’s recommendations and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Reads were mapped to the Gallus gallus genome assembly (ftp://ftp.ensembl.org/pub/release-92/fasta/gallus_gallus/dna/) using HISAT2 version 2.0.4 (Center for Computational Biology, Johns Hopkins University) (Kim et al. 2015). HTSeq version 0.9.1 (Python Software Foundation) was used to count the reads (Anders et al. 2015). The gene expression levels of the transcripts were quantified as fragments per kilobase of transcript sequence per million base pairs sequenced (fragments per kilobase million [FPKM]) (Trapnell et al. 2010). The DESeq R package version 1.18.0 (Bioconductor) (Anders and Huber 2010) was used to analyse DEGs in the heat-stressed heart samples (p < .05; false discovery rate [FDR] < .05 as a threshold). The GOseq R package (Young et al. 2010) and KOBASE software (Mao et al. 2005) were used to analyse gene ontology (GO) and the results of Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis, respectively.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) and statistical analysis

We used qRT-PCR analysis to determine the expression levels of five genes (COX6A1, UQCRB, NDUFS6, MCEE and ALDOB). The primer pairs used to amplify the selected genes are shown in Table S1. The 2−ΔΔCT method (Livak and Schmittgen 2001) was used to analyse the relative levels of gene expression determined in qRT-PCR experiments. The mRNA expression levels were analysed with one-way ANOVA using SPSS software version 19.0 (SPSS, Chicago, IL).

Data deposition

The RNA-seq analysis data have been submitted to the Sequence Read Archive (SRA) at the National Centre for Biotechnology Information (NCBI), and assigned accession number SRP152925 (https://www.ncbi.nlm.nih.gov/sra/SRP152925).

Results and discussion

The results obtained for BW, HAW and HRW are presented in Figure S1A, B and C, respectively. After heat stress treatment (38 ± 1°C, 8 h/d, for 7d), the BW of the heat stress treatment group (BW28 = 354.8 ± 34.8 g) was significantly decreased (p = .03) compared with that of the control group (BW28 = 384.8 ± 58.9 g). We
found that heat stress decreased the BW of yellow-feather broilers by 7.8%. In comparison previous studies have shown a 12.8% reduction in the BW of commercial broilers subjected to heat stress (32°C, period 2–4 weeks of age) (Geraert et al. 1996), whereas in AIL chicken lines bred for thermo-tolerance (Deeb and Lamont 2002), exposure to heat stress (35°C, 7 h/d, for 7d), resulted in a BW gain ratio (BW28–21) of 58.9% (Angelica et al. 2015). In this study, the BW gain ratio (BW28–21) in response to heat stress was 60.0% indicating that yellow-feather broilers tolerated a higher temperature (38 ± 1°C, 8 h/d, 7d) environment. A decrease in growth performance in response to heat stress has previously been shown to be associated with various changes in certain organs, including intestinal damage, and relative weight reduction in immune-related organs and the heart (Quinteirofilho et al. 2010; Zhang et al. 2017). In this study, the HAW of the heat stress treatment group (HAW28 = 2.18 ± 0.16 g) showed a significant decrease (p = .001) compared with that of the control group (HAW28 = 2.75 ± 0.11 g). In contrast, however, the HRW of the heat stress treatment group (HRW28 = 0.61 ± 0.02%) did not differ significantly (p = .538) from that of the control group (HRW28 = 0.58 ± 0.08%). These results indicated normal physiological development of the heart in yellow-feather broilers subjected to a high-temperature environment (Ven et al. 2013).

The number of total clean reads for the identified DEGs ranged from 53 to 62 million and approximately 90% of the clean reads were mapped on the chicken reference genome (Table S2), of which 78% of reads were mapped to exons (Figure S2). A total of 37 genes (FDR < 0.05) were differentially expressed in response to heat stress in the heart of yellow-feather broilers (Figure 1). In contrast, 1089 DEGs (FDR < 0.1) were identified in the heart transcriptome of Ross broilers subjected to heat stress (35–37°C, 8 h/d, for 14d) (Zhang et al. 2017). These findings indicate that the heat stress response of broilers is to a certain extent dependent on the duration of heat stress. Of the 37 DEGs identified in this study, 19 were downregulated and 18 were upregulated and the log2 (fold change) induced by heat ranged from –4.24 to 6.17.

We performed GO term enrichment analysis of the DEGs using the GOseq R package to investigate the biological processes related to heat stress. We accordingly found that the 30 most enriched biological process (BP) GO terms of the DEGs expressed in the heart are all related to energy metabolism. The heart requires large amounts of energy to maintain ionic homeostasis and contraction (Jafri et al. 2001), and in this regard, KEGG (Kanehisa and Goto 2000) pathway analysis of these DEGs revealed the enrichment of three pathways (p < .05) oxidative phosphorylation (KO: 00190), cardiac muscle contraction (KO: 04260) and carbon metabolism (KO: 01200) (Figure S3). The products of DEGs associated with the ‘oxidative phosphorylation’ pathway are: cytochrome c oxidase (COX) subunit 6A1 (COX6A1), ubiquinone oxidoreductase subunit S6

**Figure 1.** Volcano plot statistics of differentially expressed genes (DEGs) in the heat stress group (38 ± 1°C, 8 h/d, for 7 d) compared with the control group (25 ± 1°C) group in yellow-feather broilers. Y-axis: the mean log10 (FDR) gene expression value. X-axis: the log2 fold change value of gene expression. The red and green dots represent up- and downregulated DEGs, respectively (FDR < 0.05).
(NDUFS6) and ubiquinol-cytochrome c reductase-binding protein (UQCRB). Given that the heart has a high energy dependence, it is particularly susceptible to oxidative phosphorylation defects, which are considered oxidative phosphorylation diseases (Limongelli et al. 2010; Finsterer and Kothari 2014). Disruption of the function of the oxidative phosphorylation electron transport chain leads to mitochondrial dysfunction, and in this study, we found that the oxidative phosphorylation pathway was the most significantly enriched pathway, which supports this view. The interrelated oxidative phosphorylation DEGs (COX6A1, UQCRB and NDUFS6) may be involved in heat stress-related energy metabolism, and their differential expression indicates that yellow-feather broilers have specific physiologic mechanisms for regulating body growth in response to high-temperature environments.

COX is a key enzyme in cellular respiration (Brudvig and Wikström 2005). The COX6A1 gene encodes mitochondrial protein COX subunit 6A1, which drives ATP synthesis in the mitochondrial electron transport chain (Fornuskova et al. 2010). Another oxidative phosphorylation-related DEG, UQCRB, encodes subunit 7 of ubiquinol cytochrome c reductase. UQCRB plays a key role in hypoxia-induced angiogenesis and mitochondrial-mediated metabolic disorders (Jung et al. 2011; Chang et al. 2014; Chang et al. 2015), and the loss of UQCRB function inhibits angiogenesis (Cho et al. 2013). NDUFS6 encodes NADH, ubiquinone oxidoreductase and dehydrogenase (ubiquinone) Fe-S protein 6 (Loeffen et al. 1998). Defects in NADH are associated with energy generation disorders (Gabaldon et al. 2005; Spiegel et al. 2009). NADH deficiency leads to neurodegeneration, muscle weakness, cardiac failure, liver failure and early death (Kirby et al. 1999; Loeffen et al. 2000). NDUFS6 mutations cause respiratory chain disorders (Kirby et al. 2004). Among these DEGs, COX6A1 and UQCRB are also involved in the cardiac muscle contraction pathway. In this study, three DEGs were downregulated in the heat stress treatment group and we conclude that these are involved in the energy metabolism of heat stress and the physiologic response to high temperatures. The ‘carbon metabolism’ pathway includes methylmalonyl-CoA epimerase (MCEE) and fructose-bisphosphate aldolase B (ALDOB). The five selected genes associated with KEGG pathways were identified by qRT-PCR (Figure S4).

Conclusions

The findings of this study provide evidence for the transcriptomic regulation of heat stress responses in the heart of yellow-feather broiler chickens. Heat stress decreases the BW28 (p = .033) and HAW28 (p = .001) of these broilers, whereas the HRW28 was not significantly decreased (p = .538). A total of 37 DEGs related to energy metabolism were detected in heart tissues in response to heat stress. These results indicate that the heart of yellow-feather broilers has specific physiologic mechanisms for regulating body growth in response to high-temperature environments.

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Disclosure statement

We certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

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