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RNA-SEQ STUDY OF HEPATIC RESPONSE OF YELLOW-FEATHER CHICKENS TO ACUTE HEAT STRESS

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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 liver of yellow-feather broilers that had been subjected to acute heat stress treatment (38±1°C for 4 h, recovery 2 h) and determine the response of the liver to high temperature and its effects on yellow-feather broiler physiology. We found that the cloacal temperature and respiratory rate of yellow-feather chickens were significantly increased immediately after the initiation of acute heat stress (38°C) treatment. And after recovery for 2 h, there was no difference in the cloacal temperature and respiratory rate between the acute heat stress and control groups. A total of 834 DEGs were observed in response to heat stress by RNA-seq. Almost half of the DEGs were involved in the lipid and energy metabolism, including fatty acid metabolism (*ACOX1*, *ACACA*, *ACSL1*, *ACSL6*, *ACAA1*, *ACAA2*, *HADHB*, and *FASN*) and propanoate metabolism (*ACSS2*, *ALDH2*, *ACACA*, *DLAT*, *ALDH7A1*, *MDH1*, *ME1*, *ABAT*, *SUCLG2*, and *ACSS3*). Our findings provide the context for RNA-seq studies in the liver of yellow-feather chickens and suggest that the liver of yellow-feather broilers has the lipid and energy metabolism physiological mechanisms activated in response to heat stress.

Key words: yellow-feather chickens, liver, heat stress, RNA-seq

Global warming is affecting the broiler chicken industry worldwide (Windhorst, 2007). In the past few decades, broiler production performance, particularly in terms of growth rates, has been significantly improved through genetic selection (Deeb and Cahaner, 2002; Mckay et al., 2000). However, high environmental temperatures have a more detrimental effect on fast-growing broilers than on slow-growing broilers (Cahaner and Leenstra, 1992; Cheng et al., 2018; Lu et al., 2007), particularly with regards to the body weight of these chickens (Rimoldi et al., 2015; Sohail et al.,

2012). Liver, which performs important functions in maintaining the homeostasis of energy metabolism, is more sensitive than other organs to acute heat stress in broilers (Huang, 2017; Hubbard et al., 2018; Jastrebski et al., 2017; Lin et al., 2006; Tang et al., 2015; Xie et al., 2013) and is, thus, an ideal candidate tissue for studying the effects of this stress on lipid and energy metabolism.

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 flavor. Previous studies on chickens have shown that fast-growing broilers liver transcriptome responded to treatment with cyclic high ambient temperature and observed changes in metabolic, physiologic, and cellular responses (Coble et al., 2014; Jastrebski et al., 2017; Lan et al., 2016). Study of heart RNA-seq of yellow-feather chickens subjected to heat stress showed that the heart of these chickens has specific physiological mechanisms for regulating body growth under heat stress (Zhang et al., 2019). However, to date, there has been limited research on the response of yellow-feather broilers liver to heat stress. In the present study, we investigated the expression of genes in the liver of yellow-feather chickens with regards to the response to acute heat stress and analyzed the effects of heat stress on the metabolism of these birds.

Material and methods

Experimental design and management of animals

The 36 male yellow-feather chickens (65-day-old) were raised in two environmentally controlled rooms at the Poultry Breeding Center of Guangdong Ocean University, each of which was divided into three pens. Each pen contained six cocks, which were acclimated to the environment for 5 days and had access to feed and water *ad libitum*. Seventy-day-old yellow-feather chickens in one of environmentally controlled rooms were subjected to heat stress for 4 h at 38°C and 55% relative humidity (the 4HR0 group), followed by recovery for 2 h at 25°C and 55% relative humidity (the 4HR2 group). The yellow-feather chickens in another environmentally controlled room were kept at 25°C and 55% relative humidity (the control group). All the birds were cared for and treated in accordance with the guidelines provided by the Guangdong Ocean University Animal Care and Use Committee (permit number: SYXK 2014-0053).

Phenotypic measurements and sample collection

The respiratory rate of each cock was determined by counting the number of thoracic breaths per minute. The cloacal temperature of each broiler was measured using a digital thermometer (accurate to 0.1°C), until the reading was stable. The digital thermometer was inserted into the cloaca of each cock to a depth of approximately 2.5 cm. The cocks were euthanized before heat treatment (n=6, control), after four hours of acute heat 4HR0 (n=6) and after 2 hours of recovery in thermo-neutral conditions 4HR2 (n=6), and 18 samples of liver tissue were collected and stored at

−80°C until required. Three of six liver samples of each treatment were selected at random for RNA-seq.

Transcriptome profile in response to acute heat stress

We used an Illumina HiSeq 2500 platform to identify the differentially expressed genes (DEGs) in the liver samples collected from yellow-feather chickens that were subjected to acute heat stress. For sequencing, total RNA was extracted from the liver samples using TRIzol reagent (Invitrogen, catalog no. 15596026, USA), according to the manufacturer's instructions. An NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) was used to prepare the sequencing libraries, according to manufacturer's recommendations. The sequencing reads were mapped to the *Gallus gallus* genome assembly (ftp://ftp.ensembl.org/pub/release-92/fasta/gallus_gallus/dna/) using HISAT2 v2.0.4 (Kim et al., 2015). HTSeq v0.9.1 was used to count the reads (Anders et al., 2015). The gene expression levels 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 (1.18.0) (Anders and Huber, 2010) was used to analyze the DEGs in the heat stress liver samples (P-value <0.05 and false discovery rate (FDR) <0.05 were used as the threshold values). In a simulation study evaluating DESeq the DEG detection was above 80% for n=3 and log2foldchange ≥1.0 but at a cost of higher than in other tools false discovery rate (Schurch et al., 2016). The GOseq R package (http://bioinf.wehi.edu.au/software/goseq/) (Young et al., 2010) and KOBAS software (http://kobas.cbi.pku.edu.cn) (Mao et al., 2005) were used to analyze the results of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis.

Quantitative reverse transcription-polymerase chain reaction and statistical analysis

We used quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis to determine the expression levels of six genes (*HSPA2*, *ACACA, FASN, ACSL1, ACOX1*, and *ACAA1*). The sequences of the primer pairs used for amplification of the selected genes are shown in Table 1, *GAPDH* was used as a reference housekeeping gene. The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used to analyze the gene expression levels determined by qRT-PCR. The values obtained for respiratory rate, body temperature, and mRNA expression levels were analyzed using one-way analysis of variance (ANOVA) with SPSS software (v19.0, IBM, USA).

Table 1. Information on primers for qRT-PCR

Gene name	Gene ID	Forward primer	Reverse primer	Length
ACOX1	NM_001006205	attcatcctcaacagcccca	ctgagtgtagagctgagcca	103bp
ACACA	NM_205505	tgagtgccttcgagttgtga	acatcaccactgcaaacacc	109bp
ACSL1	NC_006091	gcacccttccgacaaatacc	tgcattgctaagtcacacgg	112bp
ACAA1	NM_001197288	gagaatggcagcactacagc	ctccaaccacagcaaaggac	139bp
FASN	NC_006105	ctgatgggattttgggccac	cccctccaataagcagcaag	100bp
HSPA2	NM_001006685	ttgataagggccagatccag	ttgataagggccagatccag	105bp
GAPDH	M_11213.1	tagtgaaggctgctgctgat	aaggtggaggaatggctgtc	103bp

Data deposition

The RNA-seq data were submitted to the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) under the accession number PRJNA490069 (https://www.ncbi.nlm.nih.gov/sra/PRJNA490069).

Results

Phenotypic measurements

The cloacal temperature and respiratory rate of yellow-feather chickens were significantly increased immediately after the initiation of acute heat stress (38°C) treatment ($P<0.05$; Figure 1). The average cloacal temperature reached 46°C in 4 h of acute heat stress treatment, whereas the respiratory rate began to decrease. After recovery for 2 h, there was no difference in the cloacal temperature and respiratory rate between the acute heat stress and control groups.

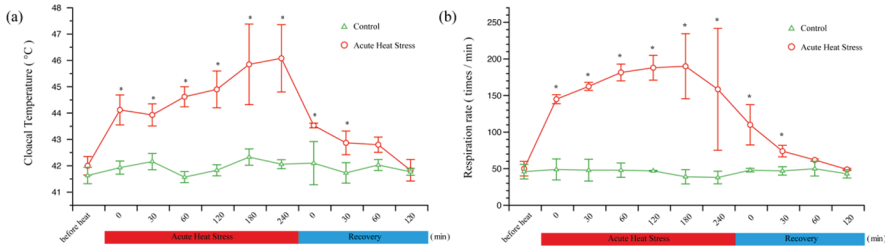

Figure 1. Cloacal temperature (a) and respiratory rate (b) of acute heat stress and control yellow-feather chickens during the 4 h heat stress and 2 h recovery periods. The asterisk indicates the significant difference of each detected time between the heat stress and control groups ($P<0.05$)

Analysis of differential gene expression

The total number of clean reads varied from 46 to 73 million, and approximately 90% of these reads were mapped onto the reference genome of chicken (Table 2). Some differences in read counts were detected between biological replicates, especially between Control_1 and 4HR2_3. The quantity of total mRNA analyzed was identical in these samples, indicating that the different read counts may have arisen during processing. However, this discrepancy did not significantly affect gene expression analysis because the FPKM values were corrected for the total number of read counts for each sample. Approximately 75% of the reads were mapped to the exons (Figure 5). A total of 834 DEGs were observed in response to acute heat stress in the liver samples of the 4HR0 and 4HR2 groups. The number of DEGs in the 4HR2 group (n = 786) was 7 times higher than that of the 4HR0 group (n = 103, Figure 2). This shows that during the period of recovery from acute heat stress, liver produces a strong response, which is manifested in the different number of DEGs under acute heat stress and recovery conditions. Cluster analysis of DEGs was used to determine the relationship between the acute heat stress and control groups (Figure 6). Of these 834 genes, 481 were down-regulated and 353 were up-regulated

(Figure 2). A total of 45 down-regulated DEGs and 10 up-regulated DEGs, respectively, were shared with the 4HR0 and 4HR2 groups. According to DEGs GO and KEGG analysis results, six DEGs (*HSPA2*, *ACACA, FASN, ACSL1, ACOX1*, and *ACAA1*) associated with fatty acid metabolism and HSP were selected for validation by qRT-PCR. The analysis of the expression levels of six DEGs results were shown in Table 3. The results of qRT-PCR were highly correlated with the FPKM values estimated by RNA sequencing (r = 0.99).

Table 2. Number of reads before and after FastQC filtering, and number of mapped and uniquely mapped reads

cDNA library	Raw reads	Clean reads	Mapped reads	Uniquely mapped
Control_1	75,770,336	73,606,478	65,859,010	62,887,732
Control _2	67,629,042	65,974,694	58,986,419	56,409,777
Control _3	60,102,230	58,707,790	53,203,729	51,025,529
4HR0_1	58,486,260	56,919,196	50,959,137	48,798,262
4HR0_2	63,741,388	62,248,222	56,567,826	54,336,101
4HR0_3	62,232,272	60,889,198	55,359,485	53,077,675
4HR2_1	64,954,228	63,327,084	56,476,671	54,254,204
4HR2_2	64,615,270	63,013,114	57,369,907	54,949,809
4HR2_3	48,202,070	46,718,578	41,835,339	40,175,531
Average	62,859,392	61,267,150	55,179,724	52,879,402

4HR0 group was subjected to acute heat stress at 38°C for 4 h, 4HR2 group was subjected to recovery for 2 h at 25°C after acute heat stress, and control group was maintained at 25°C during the experiment period. There are three biological repetitions for each group.

GO and KEGG pathway analysis of the DEGs

We performed GO enrichment analysis of the DEGs using the GOseq R package to identify the biological processes related to acute heat stress. The significantly (FDR < 0.05) enriched GO terms for the DEGs in the liver samples are represented in Figure 3. These GO terms included biological process (BP), cellular component (CC), and molecular function (MF) terms.

We detected 48 significantly enriched GO terms for the 4HR0 and 4HR2 groups. Among these, 37, 4, and 7 terms were related to BP, CC, and MF categories, respectively. The BP categories related to metabolic, catabolic, and biosynthetic processes in the liver were enriched. The metabolic processes included metabolic progress (n = 422), single-organism metabolic progress (n = 225), small molecule metabolic progress (n = 112), lipid metabolic progress (n = 71), and fatty acid metabolic progress (n = 28). The catabolic processes included organic acid catabolic process (n = 25), carboxylic acid catabolic process (n = 25), and cellular amino acid catabolic process (n = 15). The biosynthetic processes included organic acid biosynthetic process (n = 21), carboxylic acid biosynthetic process (n = 21), and small molecule biosynthetic process (n = 28). We also noticed enrichment of the oxidation–reduction process (n = 86) term. The results of GO enrichment analysis showed that in liver, 50.6% DEGs were mainly involved in metabolism, under acute heat stress. A total of 43 GO terms were found to be enriched in the 4HR2 group.

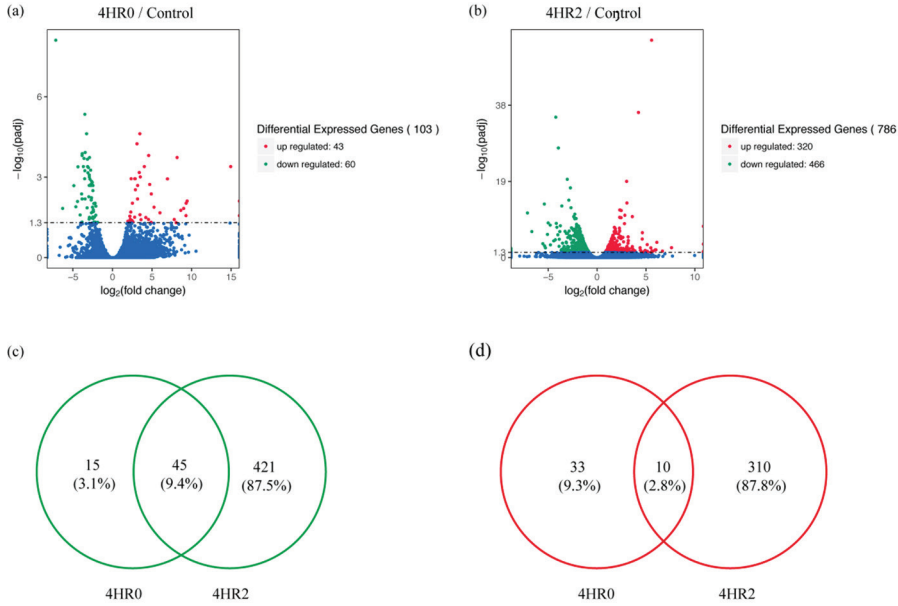

Figure 2. Volcano plot and venn diagram of differentially expressed genes (DEGs) in 4HR0 and 4HR2 groups compared with control group, respectively. Volcano plot DEGs in 4HR0 and 4HR2 group were displayed in panel a and panel b, respectively. Y-axis: the mean gene expression value of $\log_{10}$(FDR). X-axis: the log2fold change value of gene expression. The red and green dots represent up- and down-regulated DEGs, respectively (FDR<0.05). Venn diagram of up- and down-regulated DEGs were displayed in panel c and panel d, respectively. (c) There were 421 (87.5%) and 15 (3.1%) down-regulated DEGs in 4HR2 and 4HR0 group, respectively. And 45 (9.4%) down-regulated DEGs were shared with 4HR2 and 4HR0 groups. (d) There were 310 (87.8%) and 33 (9.3%) up-regulated DEGs in 4HR2 and 4HR0 group, respectively. And ten (2.8%) up-regulated DEGs were shared with 4HR2 and 4HR0 groups. 4HR0 group was subjected to acute heat stress at 38°C for 4 h, 4HR2 group was subjected to recovery for 2 h at 25°C after acute heat stress, and control group was maintained at 25°C during the experiment period

The KEGG pathway analysis (Kanehisa and Goto, 1999) of the DEGs was performed to characterize the functional consequences of changes in gene expression in the liver under acute heat stress and recovery. It was observed that the DEGs in the 4HR0 and 4HR2 groups were enriched in fatty acid metabolism (n = 5 and n = 16, respectively) and in the biosynthesis of unsaturated fatty acids (n = 4 and n = 11, respectively) (Figure 4). The steroid biosynthesis (n = 3) and caffeine metabolism (n = 2) genes were enriched in the 4HR0 group (Figure 4 A). The pathways of pyruvate metabolism (n = 11), glycine, serine, and threonine metabolism (n = 11), propanoate metabolism (n = 8), and metabolic pathways (n = 105) were enriched in the 4HR2 group. In addition, genes of glycolysis/gluconeogenesis (n = 14) and peroxisome (n = 14) were also enriched in the 4HR2 group (Figure 4 B). The results of KEGG pathway analysis also showed that DEGs were mainly involved in metabolism in liver under acute heat stress. Moreover, the biochemical pathways altered in the 4HR2 group were more complex than those in the 4HR0 group.

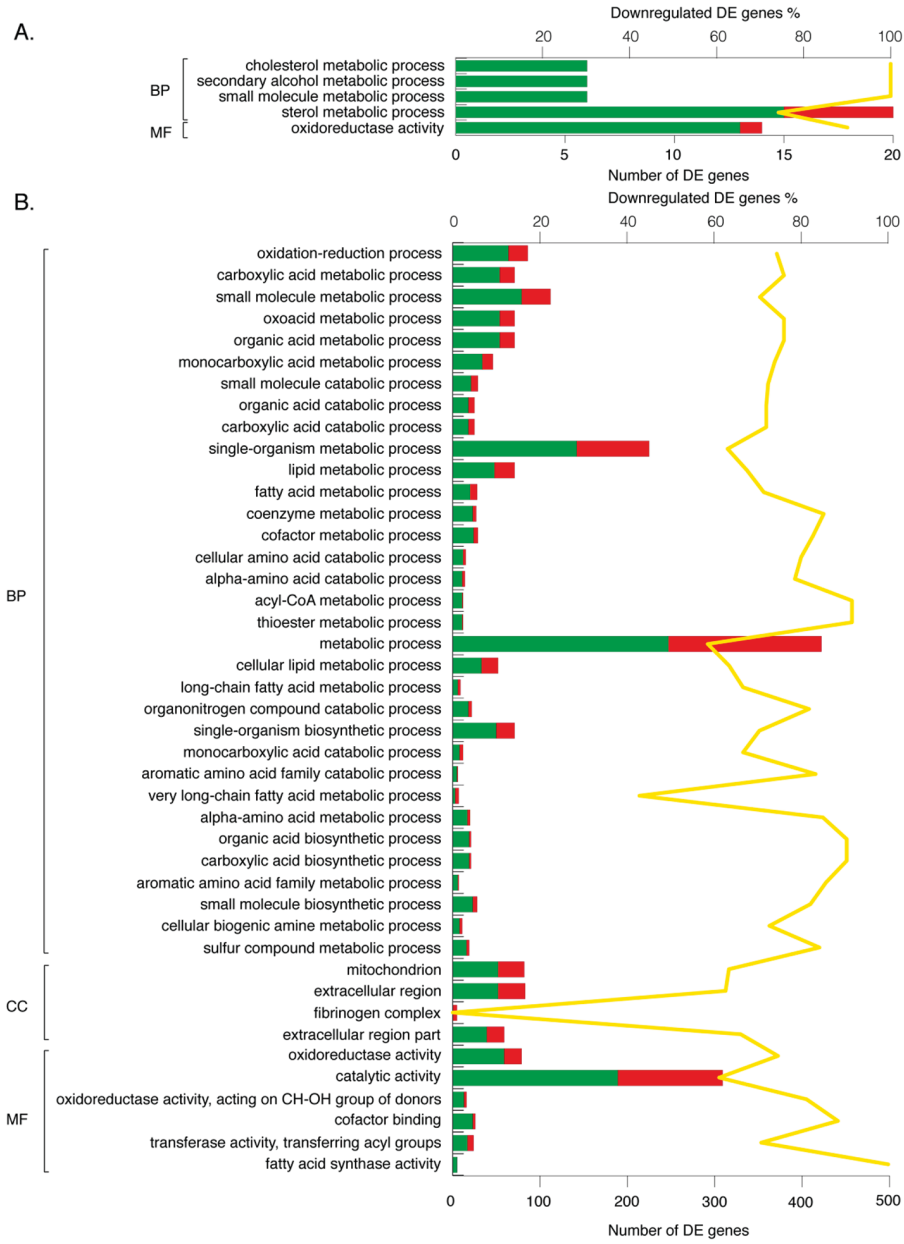

Figure 3. The histogram displaying the gene ontology (GO) terms of differentially expressed genes (DEGs) enrichment significantly (FDR<0.05) in 4HR0 (A) and 4HR2 (B) group subjected to acute heat stress. In 4HR0 group, the average of 93.6% DEGs of enrichment in GO terms were down-regulated. Most of the significant terms were in the BP category. In 4HR0 group, the average of 73.7% DEGs of enrichment in GO terms were down-regulated. Most of the significant terms were in the BP category. BP: biological processes, CC: cellular components and MF: molecular functions

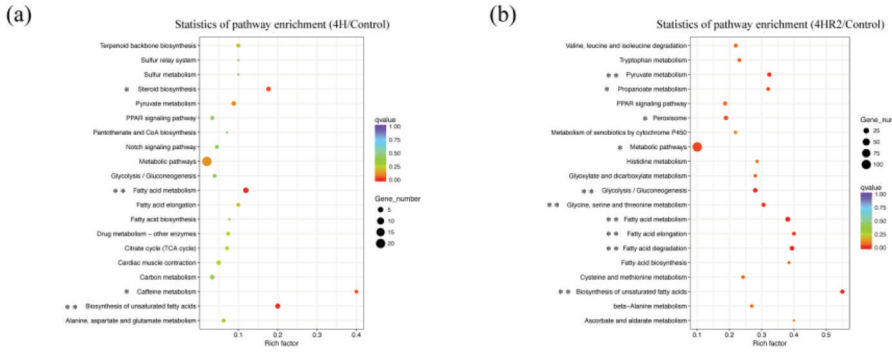

Figure 4. The KEGG pathway enriched analysis significantly (FDR<0.05) for the differentially expressed genes (DEGs). The asterisk indicates the FDR<0.05 and double asterisk indicates the FDR<0.01. Rich factor refers to the ratio of the number of differentially enriched genes to the number of annotated genes in the pathway. The rich factor represents the degree of enrichment

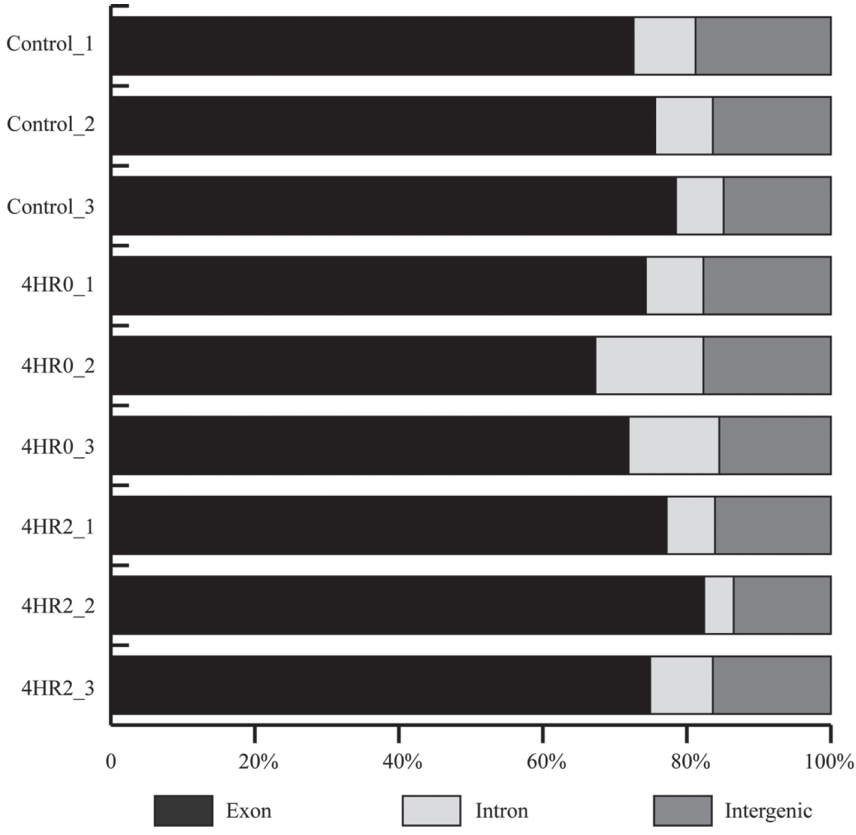

Figure 5. The clean reads were mapped to exons, introns and intergenic genes. 4HR0 group was subjected to acute heat stress at 38°C for 4 h, 4HR2 group was subjected to recovery 2 h at 25°C after acute heat stress, and control group was maintained at 25°C during the experiment period. There are three biological repetitions for each group

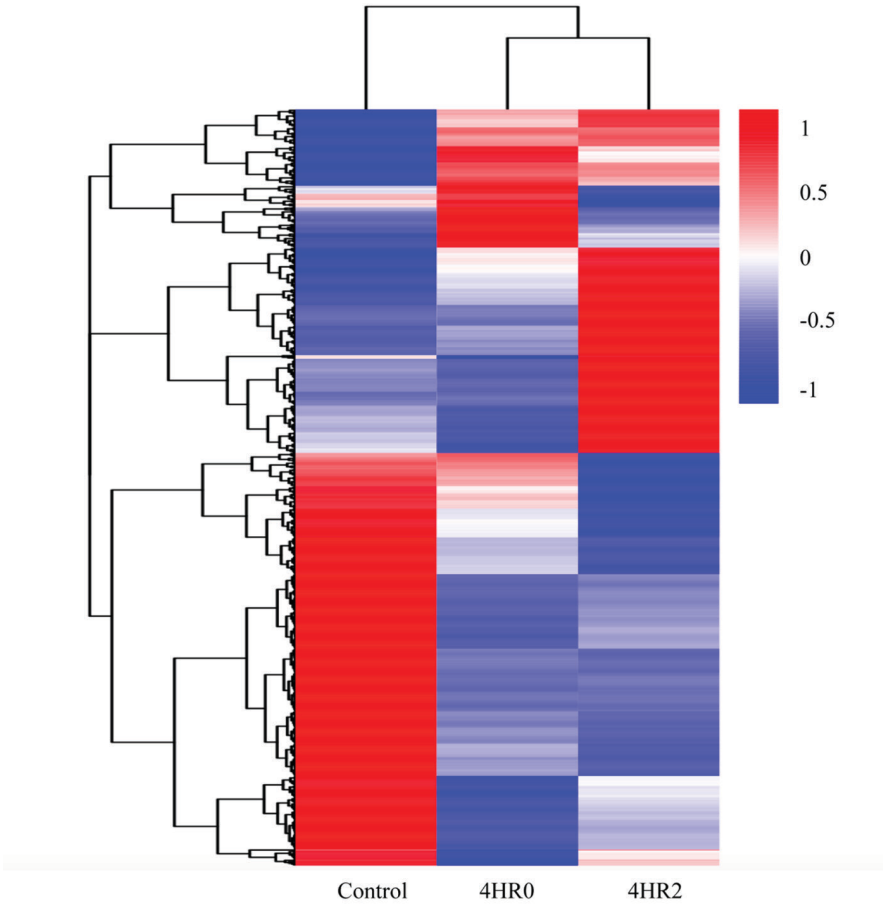

Figure 6. Cluster analysis of differentially expressed genes (DEGs). Log_{10}(FPKM+1) value normalized transformation was used for the FPKM hierarchical clustering map. Red and blue indicated higher and lower expression genes, respectively

Table 3. Validated DEGs of RNA-seq results by qRT-PCR

Gene name	Log_2fold change of RNA-seq		Log_2fold change of qRT-PCR	
	4HR0/control	4HR2/control	4HR0/control	4HR2/control
ACOX1	1.41	1.33***	1.48*	1.42*
ACACA	–1.65	–1.94***	–1.57*	–2.01**
ACSL1	2.07	2.34***	1.89*	2.25*
ACAA1	0.59	1.87***	0.45	2.13**
FASN	–1.63	–2.89***	–0.98	–3.02**
HSPA2	8.99**	2.14***	6.38**	2.46**

n=6. *P<0.05, **P<0.01, ***P<0.001. 4HR0 group was subjected to acute heat stress at 38°C for 4 h, 4HR2 group was subjected to recovery for 2 h at 25°C after acute heat stress, and control group was maintained at 25°C during the experiment period.

Discussion

In this study, the rate of change in cloacal temperature after acute heat stress was reported to be slower in yellow-feather chickens compared to that in broilers (May et al., 1987), and the temperature returned to normal after recovery for 2 h, indicating better heat tolerance of the yellow-feather chickens than the commercial broilers.

The physiological response of different breeds to heat stress was reported to be different (Wang et al., 2018). The effect of acute heat stress on the liver transcriptome of broilers was more pronounced than that of chronic heat stress (Lan et al., 2016). The results of previous studies on the liver transcriptome of broilers showed that the DEGs under heat stress were associated with apoptosis, tissue repair, cellular calcium levels (Coble et al., 2014), and immune response (Lan et al., 2016). In this study, the GO and KEGG results show that the response of yellow-feather chickens to heat stress is different from those of the commercial broilers. The response of yellow-feather chickens to heat stress was related apparently more with lipid and energy metabolisms than with physiology.

The DEGs of *ACOX1*, *ACACA*, *ACSL1*, *ACSL6*, *ACAA1*, *ACAA2*, *HADHB*, and *FASN* were associated with fatty acid metabolism. In fatty acid metabolism, *ACACA* and *FASN*, which are associated with the initiation of fatty acid metabolism, were down-regulated in the 4HR2 group. In chicken, *ACACA* encodes a key lipogenesis factor, which has a similar function in mammals (Hillgartner et al., 1996; Takai et al., 1988), and catalyzes the first committed step in the biosynthesis of fatty acids: acetyl-CoA was converted to malonyl-CoA (Abu-Elheiga and Wakil, 2005; Tong, 2005), and the inhibition of ACACA reduces *de novo* lipogenesis by attenuating the formation of malonyl CoA and long chain fatty acids (Jump et al., 2011), which might play a critical role in the formation of abdominal fat in growing chickens. *FASN* encodes a key multifunctional enzyme that contributes to the synthesis of fatty acids (Diraison et al., 2002). The mRNA of *FASN* was mainly expressed in the liver of chicken (Cui et al., 2012), and its expression level was positively correlated with the fat content in the body (Mildner and Clarke, 1991; Nogalska and Swierczynski, 2001). *FASN* knockout mice die before birth and heterozygous knockout mice die at various stages of development, and indicated that the essential role of *FASN* is highlighted in the fatty acid biosynthesis (Chirala et al., 2003). *ACSL1*, *ACSL6*, *ACOX1*, and *ACAA1* are involved in β-oxidation of fatty acids. The metabolism of fatty acids, such as the degradation of very long-chain fatty acids, occurs through the β-oxidation process in the liver (Hashimoto et al., 1999; Veldhoven, 2010). ACSL1 and ACSL6 belong to the acyl-CoA synthetase (ACS) enzyme family (Jia et al., 2007). ACSL1 and ACSL6 prefer long-chain fatty acids (Marszalek et al., 2005; Suzuki et al., 1990) and increased expression of ACSL1 gene is associated with β-oxidation in liver (Schoonjans et al., 1996). *ACSL1* was overexpressed in fast-growing chickens (Resnyk et al., 2017). ACOX1 acts as the first and rate-limiting enzyme in the β-oxidation pathway and catalyzes the oxidation of very long-chain fatty acids (VLCFAs) (Fan et al., 1996). This shows that each gene has a different function, which might lead to different fates of fatty acids (Kim et al., 2001; Van Horn et al., 2005). The up-regulation of ACOX1 was reported to stimulate he-

patic fatty acid oxidation, resulting in excess energy burning in the liver (Misra and Reddy, 2014). These DEGs were also involved in glycolysis/gluconeogenesis and peroxisome pathways. The main roles of liver are: lipid metabolism, glycolysis, and gluconeogenesis. In chickens, liver is more susceptible than other organs to oxidative stress under acute heat stress conditions (Hubbard et al., 2018; Jastrebski et al., 2017; Lin et al., 2006; Tang et al., 2015; Xie et al., 2013).

The results of KEGG analysis showed that the DEGs, *ACSS2*, *ALDH2*, *ACACA*, *DLAT*, *ALDH7A1*, *MDH1*, and *ME1* were involved in pyruvate metabolism and *ACSS2*, *ACACA*, *ABAT*, *SUCLG2*, and *ACSS3* were involved in propanoate metabolism. The pyruvate and propanoate levels were reported to be associated with energy metabolism (Armentano et al., 1991; Chen et al., 2000; Lam et al., 2005). Pyruvate decreases the intracellular accumulation of triglycerides and under conditions of negative energy balance, liver cells have decreased capacity for converting propionate to glucose (Armentano et al., 1991). Aldehyde dehydrogenases (ALDHs) represent a family of enzymes that catalyze the oxidation of aldehydes to the corresponding carboxylic acids (Vasiliou et al., 2000). ALDH2 is known to catalyze the hydrolysis of esters (Sládek, 2010) and activation of *ALDH2* attenuates hepatic injury via clearance of cytotoxic aldehydes (Zhang et al., 2018 a). The ALDH7A1 protein, also known as 'antiquitin,' might have osmoregulatory properties (Vasiliou and Nebert, 2005). And ALDH7A1 catalyzes the terminal step of lysine catabolism, the NAD+-dependent oxidation of α-aminoadipate semialdehyde to α-aminoadipate (Korasick et al., 2017). Short-chain acyl-CoA synthetase (ACSS) is involved in the initial activation step in the metabolism of short-chain fatty acids. ACSS2 and ACSS3 mRNAs are present in various tissues, including liver (Fujino et al., 2000; Yoshimura et al., 2017). The key role of ACSS2 in lipogenesis is to catalyze the synthesis of acetyl-CoA from acetate (Fujino et al., 2000). Other studies have investigated the underlying mechanism of ACSS2's role in nutrient sensing (Rui et al., 2015). The expression of *ACSS2* gene was reported to be involved in fatty acid synthesis, triacylglycerol synthesis, and fatty acid oxidation (Xu et al., 2018). Moreover, ACSS2 has a higher affinity for propionate (Ingram, 2014). The role of ACSS2 was in promoting cell growth and survival in nutrient-stressed conditions (Dodhia et al., 2017). ACSS3 is present in the mitochondrial matrix of liver and has high affinity for propionic acid, and its expression is up-regulated under ketogenic conditions (Yoshimura et al., 2017). ACSS3 is related to the propanoate metabolism, and catalyzes the conversion of propanoyl-CoA to propanoate (Buitenhuis et al., 2014). DLAT catalyzes the conversion of pyruvate to acetyl-CoA, presumably to facilitate oxidative phosphorylation, ATP generation, and catabolic reactions (Goh et al., 2015). The important roles of the essential malate dehydrogenase, i.e., mitochondrial malate dehydrogenase (MDH2), is to produce energy through aerobic respiration in the Krebs cycle. In addition, MDH2 is associated with *de novo* lipid synthesis (Bourneuf et al., 2006; Schmid et al., 2010). Knockdown of DLAT reduced cell proliferation by approximately 20–45% that presumably resulted in decrease in energy production (Goh et al., 2015). ME1 (malic enzyme) is a NADP-dependent lipase, which is associated with the conversion of L-malate to pyruvate. ME1 is linked between the glycolytic pathway and the Krebs cycle through acetyl transfers (Macdonald, 1995). Moreover,

MDH2 and ME1 are involved in the synthesis and secretion of lipids (Bourneuf et al., 2006; Zhou et al., 2012). The expression levels of *ME1* in chickens with fatty liver were higher than those in chickens without fatty liver (Zhang et al., 2018 b).

In summary, the results provide insights into the transcriptomic regulation of acute heat stress in the liver of yellow-feather chickens. A total of 834 DEGs were detected under heat stress. These were found to be involved in the lipid and energy metabolism, including fatty acid metabolism, pyruvate metabolism, and propanoate metabolism. Our findings provide the context for RNA-seq studies in the liver of yellow-feather chickens and suggest that the liver of yellow-feather broilers has the lipid and energy metabolism physiological mechanisms activated in response to heat stress.

Acknowledgments

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Conflict of interest

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

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