RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Special Issue: 36-55 (November 2025)

Resveratrol Modulates Intestinal Structure and Function in Chickens Exposed to Heat Stress

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ABSTRACT

Background: Heat stress causes oxidative damage in chicken intestinal epithelial cells, resulting in economic losses in poultry production. The potential of resveratrol to alle*via*te oxidative stress and intestinal injury in poultry was investigated.

Methods: A total of 300 one-day-old chickens were randomly assigned to five different experimental groups. The control group was maintained under normal temperature, while the remaining four groups were exposed to high-temperature conditions and received a basal diet supplemented with resveratrol for a period of 15 days. Intestinal tissue structure and serum antioxidant indices were measured. Mechanistically, transcriptome sequencing was employed to investigate gene expression changes in the jejunal mucosa. Finally, analyses of gene ontology (GO) and KEGG pathways were conducted.

Result: The results indicate that heat stress negatively affects the structure of intestinal villi, leading to increased mRNA expression of HSPB1, HSP70 and HSP90. However, the daily administration of resveratrol at a dose of 400 mg/kg of body weight reduces the excessive expression of these heat shock protein mRNAs (P<0.05). Chicks that were supplemented with resveratrol exhibited significantly elevated serum levels of SOD, CAT, T-AOC and GSH-PX (P<0.05). Transcription profiling showed 181 significantly altered differentially expressed genes (DEGs). The outcomes indicated that the DEGs in the resveratrol-treated group were primarily enriched in the VEGF, ErbB and Toll-like receptor signaling pathways. All in all, resveratrol demonstrates significant potential in alle*via*ting the adverse effects of heat stress on broilers.

Key words: Chicken, Heat stress, Intestine, Resveratrol, Transcriptome.

INTRODUCTION

High summer temperatures are a critical stressor that significantly impact the poultry industry. When the ambient temperature goes beyond the thermoneutral zone of poultry, heat stress (HS) occurs. Broiler chickens are more susceptible to HS compared with other domestic animals (Smith and Gregory, 2013; Hridoy *et al.,* 2021). In broiler chickens, HS mainly leads to problems such as reduced performance and survival rates, along with metabolic disorders, immunosuppression and deterioration of meat quality (Quinteiro *et al.,* 2010). These issues are mainly caused by oxidative damage induced by HS, which leads to a redox imbalance (He *et al.,* 2020). It has been demonstrated that HS can induce oxidative damage to intestinal epithelial cells trigger inflammatory responses and disrupt the balance of the intestinal microecology (Nanto-Hara *et al.,* 2020). Therefore, preserving the integrity of the intestinal barrier and enhancing its antioxidant capacity are crucial objectives for alle*via*ting HS-induced damage.

Oxidative stress may lead to an excessive accumulation of reactive oxygen species (ROS), potentially impairing cellular function through the damage of critical biomolecules, including DNA, proteins and lipids. This damage can eventually result in cellular malfunction and contribute to various pathological conditions (Oke *et al.,* 2024). Major events linked to heat stress involve the excessive production of heat shock proteins (HSPs) (Wang and Edens, 1998). These proteins protect cells by enhancing their tolerance and survival in stressful conditions and are considered key features of the heat shock response (Hidayat *et al.,* 2020).

Various approaches have been documented to reduce the negative impacts of heat stress on poultry farming (Sumanu *et al.,* 2022; Ding *et al.,* 2023). Among these, dietary supplements stand out as a simple, rapid and cost-effective solution and recent studies have shown that supplementation with biologically active natural antioxidants is a promising approach to mitigate the detrimental effects of free radicals produced during HS (Humam *et al.,* 2021; Meng *et al.,* 2022).

Resveratrol (3,4′,5-trihydroxystilbene, RES) is a naturally bioactive polyphenol that exists in various plants,

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How to cite this article: Shen, X., Liu, X., Wang, G., Liao, J., Yu, S., Wang, L. and Cheng, B. (2025). Resveratrol Modulates Intestinal Structure and Function in Chickens Exposed to Heat Stress. *Indian Journal of Animal Research*. **59 (Special Issue):** 36-55. doi: 10.18805/IJAR.BF-2050.

Submitted: 23-07-2025 **Accepted:** 17-09-2025 **Online:** 27-11-2025

including peanuts, grapes and berries (Meng *et al.,* 2022). It has garnered significant attention for its antioxidant and anti-inflammatory properties (Ammari *et al.,* 2025). Resveratrol exhibits multiple biological activities, such as reducing oxidative stress, regulating immune responses and promoting cell proliferation (Ding *et al.,* 2023; Greeshma *et al.,* 2024). Resveratrol not only inhibits the formation of glutathione disulfide but also maintains glutathione in its active, reduced state, thereby protecting cells from oxidative damage. Furthermore, it modulates inflammatory mediators such as tumor necrosis factor-alpha and attenuates inflammatory reactions triggered by HS (Palacz-Wrobel *et al.,* 2017). Additionally, resveratrol can modulate cellular signal transduction pathways, activating the SIRT1 signaling pathway, enhancing cell energy metabolism, delaying cellular aging and improving the body's ability to adapt to stress (Ciccone *et al.,* 2022). In the poultry industry, resveratrol can improve production performance and significantly enhance resistance to the adverse effects of HS (Liu *et al.,* 2014; Zhang *et al.,* 2017). Although numerous investigations have verified the benefits of resveratrol, the mechanism by which it affects HS requires further investigation.

In this research, we investigated how dietary resveratrol affects antioxidant capacity and intestinal development in chicks experiencing heat stress. The gut transcriptome of heat-stressed chicks in relation to resveratrol was also analyzed in order to uncover the underlying molecular mechanisms. This study enhances the application potential of resveratrol in poultry production, contributing to the sustainable development of the industry.

MATERIALS AND METHODS

Birds and management

The research protocols and animal handling procedures were reviewed and approved by the Animal Care and Use Committee of Leshan Normal University. A total of 300 one-day-old male Ros308 broiler chickens were obtained and randomly divided into five experimental groups. Each group was subjected to six replicates, with each replicate comprising ten birds. The broilers were provided with a basal diet and had free access to water at all times. This diet was formulated based on the nutritional specification for Ross308 broilers (Table 1). The normal temperature control group (NT) was kept at 25±2°C for 24 h d-1. The other four groups were exposed to a daytime temperature of 37±2°C (from 9:00 AM to 5:00 PM) and 30±2°C at night throughout the experimental period, this experiment was conducted at the research institute of Leshan Normal University.

Experiment design and sample collection

Following a 30-day acclimatization phase for all groups, varying doses of resveratrol were administered to the four treatment groups, while the control group (NT) remained untreated. From days 31 to 45, four groups were maintained in a heat-stress temperature cycle and were given a basic diet with added resveratrol (Aladdin, #R107315) in amounts of 0, 200, 400, or 600 mg per kilogram of body weight (HS, HS+Res200, HS+Res400 and HS+Res600).

At 46 days of age, the birds were humanely euthanized to collect samples. Blood samples were obtained from the wing veins (1 sample/replicate, 6 replicates/treatment). The bird specimens were subsequently euthanized before collection of intestinal tissue samples (duodenum, jejunum and ileum). Transcriptome sequencing was partially supported by Hangzhou Lianchuan Biotechnology Co., Ltd. The small intestine was partially fixed with a 4% paraformaldehyde solution, while the remaining portion was stored at -80°C for gene expression analysis.

Serum antioxidant assessment

The serum total antioxidant capacity (T-AOC), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) enzyme activities, malonaldehyde (MDA), glutathione peroxidase (GSH-Px) and thioredoxin peroxidase (TPx) levels were measured using purchased kits (Sinobestbio,

Table 1: Basic diet composition and nutritional components.

Dietary raw material composition (%)		Nutrient composition (%)	
Yellow corn	56.00	Metabolizable energy, Kcal/Kg	3,042.00
Soybean meal	34.86	Crude protein	23.30
Wheat bran	3.50	Ether extract	4.28
Corn oil	1.80	Crude fiber	2.64
Mineral meal	1.00	Calcium	0.97
Calcium dibasic phosphate	1.80	Available phosphorus	0.47
Salt	0.30	Lysine	1.38
DL-methionine	0.18	Methionine	0.60
Lysine HCl, 78%	0.16		
Premix*	0.40		
Total	100.00		

Note: *Premix offers the following vitamins and minerals per Kg: vitamin A, 9800 IU; vitamin D_3, 3850 IU; vitamin E, 50 IU; vitamin K, 1.95 mg; vitamin B_1, 1.3 mg; vitamin B_2, 10.5 mg; vitamin B_6, 7 mg; vitamin B_{12}, 35 μg; nicotinic acid, 70 mg; pantothenate, 23 mg; biotin, 0.18 mg; and choline, 1200 mg. It also provides Cu, 6.4 mg; Fe, 80 mg; Zn, 80 mg; I, 0.56 mg; Mn, 96 mg and Se, 0.24 mg.

Shanghai, China). The sample's T-AOC was quantified in terms of the concentration of standard antioxidant equivalents required to achieve an equivalent change in absorbance (ΔA). The nitroblue tetrazolium (NBT) photochemical reduction method at 560 nm was employed to determine the activity of superoxide dismutase (SOD). Using the H_2O_2 ultraviolet absorption method at 240 nm, the activity of catalase (CAT) was determined. The peroxidase (POD) activity was determined using the guaiacol oxidation method at a wavelength of 470 nm. At 340 nm, the activity of GSH-Px was measured by quantifying the oxidation rate.

Measurements of intestinal morphology

The intestinal segments were fixed with 4% paraformaldehyde, embedded in paraffin and stained with hematoxylin and eosin (H and E). The villus height and crypt depth were measured using a digital camera microscope in conjunction with SlideViewer 2.5 software. Ten intact villi and crypts were randomly selected from each sample for analysis. The height of the villi, the depth of the crypts and their ratio (V/C) were calculated.

RNA library and RNA-seq

Total RNA was isolated from jejunal tissue samples using Trizol reagent (Thermo Fisher, USA). Following RNA isolation, mRNA was fragmented utilizing the RNA Library Prep Kit (NEB, Cat# E6150). The fragmented mRNA was then converted into cDNA through reverse transcription and subsequently used to synthesize the second strand of DNA. Sequencing was performed using the Illumina NovaSeq 6000 platform. Genes with a false discovery rate (FDR) of less than 0.05 were considered to be statistically significant. Moreover, genes showing a minimum absolute fold change of 2 were classified as differentially expressed genes (DEGs). Based on the differentially expressed gene data obtained through sequencing, we carried out functional enrichment analysis utilizing Gene Ontology (GO) and conducted pathway analysis with the Kyoto Encyclopedia of Genes and Genomes (KEGG).

GO and KEGG analysis

The GO represents an international standard for classifying gene functions. The count of DEGs linked to every GO term was determined and hypergeometric tests were conducted relative to the genomic background to identify significantly enriched terms. KEGG, an integrated public database related to pathways, was utilized. Among the genes exhibiting differential expression, the KEGG database was utilized to detect metabolic and signaling pathways that were significantly enriched.

RT-qPCR analysis

RT-qPCR was employed to confirm the DEGs detected through RNA-seq. Fifteen DEGs were selected based on the alterations in their expression levels in the treated cells compared to those in the control cells. Primers specific to these genes were designed according to reference UniGene sequences. These primers are listed in Table 2 and were synthesized commercially by Tsingke Biotechnology Corporation (Beijing, China). The relative expression level of the gene was determined by the threshold cycle (Ct) method and the analysis was conducted using the comparative $2^{-\Delta\Delta Ct}$ formula. An internal control, the β-actin (ACTB) gene, was employed as an internal control to normalize the expression levels of the target genes.

Statistical analysis

A t-test was performed to assess the influence of temperature environmental factors on NT and HS samples that were not exposed to resveratrol, as well as the effects of resveratrol on HT samples administered with 400 mg/kg of the compound. To investigate the variations between treatments involving different concentrations of resveratrol (0, 200, 400, or 600 mg/kg) within the HT group, Duncan's multiple range test was utilized. A *P*-value less than 0.05 is considered to indicate a significant difference between the samples, while a P-value lower than 0.01 indicates a higher level of significance.

Table 2: Sequences of the primers used for the determination of gene expression levels.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Product size (bp)	Accession no.
HSPB1	TTCGACCAGTCCTTCGGGAT	GGGCAGCAGACGGAAGTATC	93	NM_205290.2
HSP70	CCGTGGAGTTCCTCAGATCG	TTTGCTAAGGCGACCCTTGT	133	NM_001006685.2
HSP90	GTTGAGGGTGTAGCGCGTTT	CTGGAAGGCAAAGGTCTCCA	130	NM_001109785.2
GLDC	TCGTGCGGTACATGAAGAGG	AGCTTGATCCAAGGGCACAA	164	NM_204322.3
MLN	CTTCACCCAGAGCGACATCC	TGTCCTGTAACACCTCCGTG	245	NM_001397618.1
TJP3	CATCCCCTAAGACCTCCCCA	CTGGCCTTCACAAACTGCAC	241	XM_015299758.4
SRSF2	GAGCCCTCCACCAACTTCAA	ATGCCAGGGGTCTCTCCAAT	195	NM_001001305.4
ACE	GCTTCTTATCGGCGAGGGGA	GCTGTTGTACTCACTGGCGA	242	NM_001167732.2
TLR3	CAGTAGGTTCCTGTGTCCCG	TTTCAGCTCTTCCAGTGCGT	98	NM_001011691.4
GYS2	ATCAAGGGGTACCTTGGCCT	GCTTCTGCATGCTGGCTTTT	88	NM_001406729.1
GRM1	CACGAGGGTGTCCTCAACATT	AAATCCAGCAGCAGCTCACT	139	XM_004935585.5
β-actin	CTCTGACTGACCGCGTTACT	CATTGTCAACAACGAGCGCA	70	NM_205518.2

RESULTS AND DISCUSSION

Jejunal mucosal mRNA expression

To determine the optimal dosage of resveratrol in chick feeding experiments, we employed RT-qPCR analysis to assess the expression levels of HSPB1, HSP70 and HSP90 genes in the jejunal tissue. The results demonstrated that, following 30 days of high-temperature exposure, administering resveratrol at doses of 200, 400, or 600 milligrams per kilogram effectively reduced the relative mRNA expression levels of heat shock proteins in jejunum tissue. Notably, the administration of a 400 mg/kg dose significantly suppressed the expression levels of HSPB1, HSP70 and HSP90 (P<0.05). The addition of 200 mg/kg markedly altered the expression of HSPB1 (P<0.05), while the addition of 600 mg/kg led to a substantial decrease in the expression of both HSPB1 and HSP70 (P<.05) (Fig 1).

Antioxidant biomarkers

To explore the influence of resveratrol on the overall antioxidant capacity of heat-stressed chicks, several key serum indicators were examined following supplementation with 0 and 400 mg/kg of resveratrol. The results demonstrated that chickens exposed to thermal stress exhibited significantly reduced activities of SOD, T-AOC, CAT, POD and GSH-Px compared with those maintained under normal temperature conditions (P<0.01). Compared with the normal temperature group, the serum MDA level in the HS group was significantly increased (P<0.01). During the heat stress period, supplementing the diet with 400 mg/kg body weight of resveratrol led to a notable decrease in serum MDA levels (P<0.05). Moreover, dietary addition of resveratrol improved the enzymatic activities of SOD, T-AOC, CAT, POD and GSH-Px in the serum of chickens (P<0.05) (Table 3). These findings suggest that resveratrol effectively mitigates lipid peroxidation in chickens under heat stress and promotes the activities of antioxidant enzymes in serum.

Changes in intestinal morphology

The H and E staining was conducted to examine the development of intestinal villi and crypts. In the HS group, loss or shortening of jejunal villi, degeneration of mucosal epithelial cells and villus atrophy were observed. Under high-temperature conditions, edema of the ileal mucosal villi and shedding of the apical epithelial villi were observed. The duodenal and jejunal villi of chickens under HS condition supplemented with resveratrol also exhibited mild shedding and breakage. However, the intestinal tissue structure was more intact, with less villus damage and neatly arranged cell layers (Fig 2).

The results from the paraffin sections clearly indicate that, compared with the NT group, exposure to high temperature leads to a significant reduction in villus height across all segments of the small intestine (P<0.05). In addition to structural damage and shortening of the villi, the ratio of villus height to crypt depth in each intestinal segment was also significantly decreased (P<0.05). Furthermore, a significant increase in crypt depth was observed specifically in the jejunum (P<0.05). However, under heat stress conditions, resveratrol supplementation

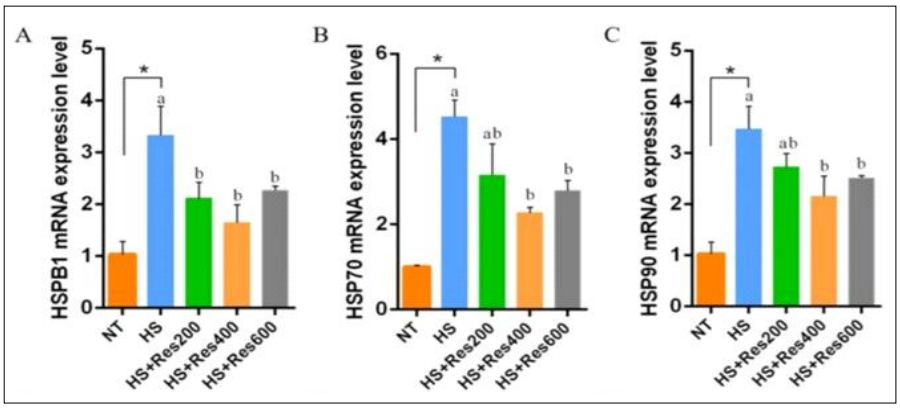


Fig 1: The mRNA expression of HSPs in the jejunal mucosa.

Table 3: Effects of resveratrol on blood biochemical indices of chickens after heat stress treatment.

Serum antioxidants	(Normal temperature) NT	(High temperature environment)		P1-value	P2-value
		HS	HS+ Res400		
T-AOC (U/mL)	36.78±0.74	22.56±1.31	27.34±0.83	0.000	0.006
SOD (U/mL)	39.93±1.05	18.87±0.41	24.36±2.33	0.000	0.016
CAT (nmol/min/mL)	565.51±12.83	415.31±9.42	528.88±2.90	0.000	0.000
POD (U/mL)	3354.54±275.76	2316.07±180.79	2939.35±65.55	0.000	0.005
MDA (nmol/mL)	7.97±0.28	11.30±0.47	10.29±0.20	0.000	0.027
GSH-Px (nmol/min/g)	196.25±4.50	101.52±6.54	119.56±5.15	0.001	0.020
TPX (nmol/min /mL)	134.20±6.68	102.00±4.99	108.29±7.21	0.003	0.281

did not induce significant changes in villus height or crypt depth across intestinal tissues (Table 4).

RNA sequencing information

To elucidate the mechanisms by which resveratrol regulates intestinal development, eight transcriptome libraries were constructed from jejunal tissue samples obtained from both the blank control group and the 400 mg/kg resveratrol-treated group. This was done to detect differences in gene expression profiles between untreated chickens and those treated with resveratrol. Using high-throughput sequencing technology, we identified a total of 181 differentially expressed genes in the jejunal tissues of chickens exposed to a high-stress environment. The list of these differentially expressed genes can be found in Supplementary File S1. The transcriptome analysis revealed that, following heat stress, 89 genes exhibited increased expression, while 92 genes showed decreased expression in the HS+Res400 group compared to the control HS group (Fig 3A). The x-axis and y-axis were plotted using multiple log2 (fold change) and -log10 (q-value) respectively, visually presenting all the genes in the differential expression analysis (Fig 3B).

The differential gene cluster heatmap intuitively displays the gene expression patterns across four samples from each of the two groups. In this heatmap, the samples are represented on the x-axis, whereas the y-axis corresponds to the selected differentially expressed genes (DEGs). Specifically, Fig 3C only includes the top 100 genes with the smallest q-values for heatmap visualization. The results show that the DEGs were clustered into two distinct groups in the heatmap, suggesting that resveratrol supplementation following heat stress significantly altered gene expression in chicken intestinal tissue at the transcriptional level (Fig 3C).

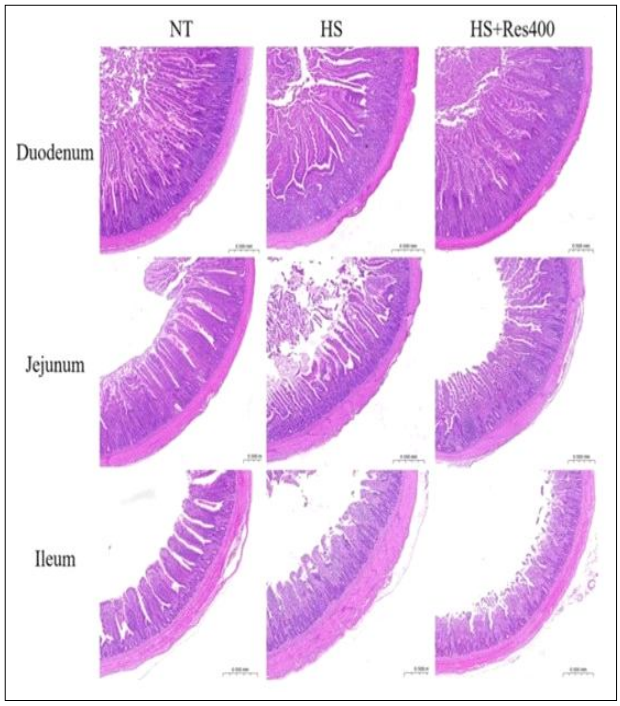


Fig 2: Effects of resveratrol supplementation on intestinal development of chickens after heat stress treatment.

Analysis of GO annotation and enrichment in KEGG pathway

The results of Gene Ontology (GO) annotation indicated that, in contrast to the control HS group, the administration of resveratrol resulted in differentially expressed genes (DEGs) predominantly associated with signal transduction, G protein-coupled receptor signaling, the NF-κB signaling pathway, transmembrane transport processes and the positive regulation of RNA polymerase II-mediated transcription (Fig 4). A list of all GO terms is provided in Supplementary File S2.

KEGG pathway-based analysis provides valuable insights into the functional roles of genes and their interactions. Fig 5 is a KEGG hierarchical bar chart in which the x-axis standing for the number of DEGs in each pathway, the y-axis lists the pathway names and the color indicates

Table 4: Effects of resveratrol supplementation on chicken intestinal development after HS treatment.

Items	(Normal temperature) NT	(High temperature environment)		P1-value	P2-value
		HS	HS+ Res400		
Duodenum					
Villus height (μm)	1257.97±67.77	1063.77±87.87	1212.33±83.54	0.039	0.101
Crypt depth (μm)	406.17±20.14	467.97±57.33	437.77±19.68	0.153	0.437
Villus height/crypt depth	3.10±0.25	2.29±0.25	2.78±0.28	0.016	0.090
Jejunum					
Villus height (μm)	959.67±96.33	689.07±76.33	814.93±119.73	0.019	0.200
Crypt depth (μm)	278.70±44.47	363.10±21.69	298.10±37.13	0.042	0.059
Villus height/crypt depth	3.51±0.78	1.90±0.26	2.77±0.59	0.027	0.078
Ileum					
Villus height (μm)	513.60±14.82	419.87±47.54	441.77±40.83	0.031	0.578
Crypt depth (μm)	174.33±11.15	205.70±18.79	194.97±34.88	0.068	0.663
Villus height/ crypt depth	2.95±0.12	2.06±0.34	2.30±0.33	0.022	0.034

Supplementary File S1: Top 100 differentially expressed genes between resveratrol-treated and control groups.

FPKM. Resveratrol_3	FPKM. Resveratrol_4	FPKM. control_1	FPKM. control_2	FPKM. control_3	FPKM. control_4	log2(fc)	pval	qval	Regulation	Significant
4.60	4.39	1.46	2.43	1.71	1.63	1.34	0.00	0.00	Up	Yes
1.40	2.33	14.08	8.79	10.73	10.69	-2.33	0.00	0.00	Down	Yes
14.23	10.16	3.25	1.71	2.38	2.21	2.14	0.00	0.00	Up	Yes
0	0	0	0.89	0	0	-11.12	0.00	0.00	Down	Yes
0.36	0	0	0	0	0	9.80	0.00	0.00	Up	Yes
0	0	2.78	0	0	0	-12.76	0.00	0.00	Down	Yes
1.77	0	0	0	0	0	12.11	0.00	0.00	Up	Yes
0	0.26	0	0	0	0	9.36	0.00	0.00	Up	Yes
0	0	0.37	0	0	0	-9.87	0.00	0.00	Down	Yes
0	0	0	1.03	0	0	-11.33	0.00	0.00	Down	Yes
0.47	2.56	3.49	18.90	29.67	21.72	-3.80	0.00	0.00	Down	Yes
0.26	0	0	0	0	0	9.32	0.00	0.00	Up	Yes
0	0	0.01	1.60	2.34	2.41	-13.96	0.00	0.00	Down	Yes
0	0	0	0	0	0	8.95	0.00	0.00	Up	Yes
0.28	0.43	3.51	5.64	2.20	2.78	-2.67	0.00	0.00	Down	Yes
0	0	0	0	0	0	9.24	0.00	0.00	Up	Yes
10.16	21.20	36.79	56.03	46.38	46.13	-1.58	0.00	0.00	Down	Yes
4.09	4.40	0.68	1.49	1.53	1.27	1.62	0.00	0.00	Up	Yes
2.47	5.06	1.23	2.69	0.97	1.39	1.43	0.00	0.00	Up	Yes
0.16	0.51	1.64	1.66	1.63	1.60	-1.98	0.00	0.00	Down	Yes
52.50	37.32	25.76	25.00	20.72	21.69	1.07	0.00	0.00	Up	Yes
0.11	0.05	0	0	0	0	9.27	0.00	0.00	Up	Yes
0.99	1.48	2.49	3.87	3.23	3.09	-1.39	0.00	0.00	Down	Yes
0.80	2.09	16.07	19.49	6.61	9.08	-2.59	0.00	0.00	Down	Yes
4.40	15.08	1.70	4.18	1.16	4.07	2.11	0.00	0.00	Up	Yes
11.42	0.01	0.01	0.01	0.01	0.02	8.48	0.00	0.00	Up	Yes
1.01	3.04	0.97	1.23	1.35	1.12	1.15	0.00	0.00	Up	Yes
1.33	0.28	0.01	0.02	0.04	0.03	4.39	0.00	0.00	Up	Yes
0.85	0.78	0.29	0.30	0.34	0.55	1.35	0.00	0.00	Up	Yes
0	0	0.19	0.32	0.58	0.38	-11.84	0.00	0.00	Down	Yes
0.20	0.40	0.95	0.96	0.88	0.95	-1.51	0.00	0.00	Down	Yes
3.55	5.83	3.48	3.01	1.88	1.65	1.01	0.00	0.00	Up	Yes
12.13	13.09	24.52	37.95	34.37	31.19	-1.22	0.00	0.00	Down	Yes
0.15	0.44	0.93	1.55	1.18	1.14	-1.80	0.00	0.00	Down	Yes
8.38	12.98	21.96	28.50	24.36	25.86	-1.10	0.00	0.00	Down	Yes
2.78	0.62	0	0	0	0	13.26	0.00	0.00	Up	Yes

Supplementary File S1: Continue...

Supplementary File S1: Continue...

0	0	0.05	0.40	0.19	0.18	-10.99	0.00	0.00	Down	Yes
0.37	0.56	0.18	0.20	0.09	0.11	1.53	0.00	0.00	Up	Yes
41.83	38.08	20.51	5.70	17.28	19.90	1.24	0.00	0.00	Up	Yes
1.54	2.32	3.87	5.18	6.49	5.21	-1.24	0.00	0.00	Down	Yes
0	0.04	0.32	1.01	0.31	0.32	-5.72	0.00	0.00	Down	Yes
0.88	1.21	2.01	2.63	3.07	2.67	-1.31	0.00	0.00	Down	Yes
783.83	345.31	250.03	245.25	154.79	183.03	1.17	0.00	0.00	Up	Yes
0.21	0.49	1.03	1.32	1.29	1.45	-1.65	0.00	0.00	Down	Yes
0	0	13.42	6.76	2.06	4.03	-5.11	0.00	0.00	Down	Yes
2.74	2.74	1.20	1.00	1.06	1.29	1.16	0.00	0.00	Up	Yes
1.42	2.33	4.16	5.94	4.07	4.47	-1.16	0.00	0.00	Down	Yes
0.91	1.15	2.13	2.33	2.41	2.12	-1.08	0.00	0.00	Down	Yes
0.37	0.77	1.58	2.12	2.04	1.93	-1.41	0.00	0.00	Down	Yes
1.15	1.21	2.63	3.53	3.65	2.89	-1.21	0.00	0.00	Down	Yes
59.88	54.19	26.18	7.28	33.32	27.03	1.25	0.00	0.00	Up	Yes
0.04	0.06	0.16	0.18	0.24	0.26	-2.07	0.00	0.00	Down	Yes
2.78	3.04	6.18	8.69	6.30	6.60	-1.08	0.00	0.00	Down	Yes
0.89	1.41	2.18	4.66	3.52	3.32	-1.55	0.00	0.00	Down	Yes
3457.70	1463.51	1010.05	923.44	863.63	983.43	1.06	0.00	0.00	Up	Yes
0.18	0.17	0.65	1.24	0.50	0.64	-2.17	0.00	0.00	Down	Yes
2.73	3.76	9.78	20.35	6.05	10.67	-1.72	0.00	0.01	Down	Yes
1.33	0.40	0.11	0.12	0.06	0.05	2.71	0.00	0.01	Up	Yes
0.83	0.29	0.02	0.08	0.02	0.02	3.29	0.00	0.01	Up	Yes
3.59	0.35	0.10	0.11	0.05	0.18	3.36	0.00	0.01	Up	Yes
0.33	0.68	1.68	1.61	2.38	1.86	-1.47	0.00	0.01	Down	Yes
1.10	1.47	2.66	4.06	5.30	4.09	-1.49	0.00	0.01	Down	Yes
1061.15	1003.83	444.66	327.36	276.69	478.72	1.01	0.00	0.01	Up	Yes
2.30	3.39	5.41	8.05	6.80	6.01	-1.05	0.00	0.01	Down	Yes
0	0	0.26	0.66	0.62	0.78	-12.50	0.00	0.01	Down	Yes
3.45	0.76	0.05	0.34	0.17	0.24	2.69	0.00	0.01	Up	Yes
0.56	0.24	0.08	0.12	0.06	0.12	1.60	0.00	0.01	Up	Yes
0.60	0.35	0	0	0	0	12.26	0.00	0.01	Up	Yes
0	0	0.52	0.33	0.43	0.46	-12.09	0.00	0.01	Down	Yes
1.69	0.46	0.08	0.17	0.14	0.20	2.25	0.00	0.01	Up	Yes
0.14	0.08	0.01	0.01	0	0	4.96	0.00	0.01	Up	Yes
0.06	1.03	0.10	0.06	0.05	0.05	3.98	0.00	0.01	Up	Yes
2.96	0.80	0.23	0.09	0.18	0.11	2.82	0.00	0.01	Up	Yes
0	0.02	1.88	0.05	0.02	0.66	-5.38	0.00	0.01	Down	Yes

Supplementary File S1: Continue...

Supplementary File S1: Continue...

0	0.19	8.81	2.45	0.19	4.35	-5.70	0.00	0.01	Down	Yes
0	0	0.26	0.22	0.16	0.25	-4.99	0.00	0.01	Down	Yes
0.73	0.76	1.68	1.83	1.87	1.84	-1.25	0.00	0.01	Down	Yes
0.20	0.06	0.74	0.87	1.04	0.65	-2.42	0.00	0.01	Down	Yes
1.34	1.57	2.87	3.76	3.80	3.24	-1.08	0.00	0.01	Down	Yes
0.91	1.10	2.15	2.38	2.70	1.89	-1.22	0.00	0.01	Down	Yes
0.99	0.16	0.03	0.08	0.05	0.12	2.39	0.00	0.01	Up	Yes
2.01	2.32	4.49	6.87	5.75	5.66	-1.08	0.00	0.02	Down	Yes
1.06	2.04	3.55	4.69	6.36	4.06	-1.32	0.00	0.02	Down	Yes
1.61	1.76	3.00	4.10	4.72	3.70	-1.28	0.00	0.02	Down	Yes
0.04	0.04	0	0	0	0	8.25	0.00	0.02	Up	Yes
0.11	0.05	0.02	0.01	0.01	0.02	1.99	0.00	0.02	Up	Yes
0	0	0.25	0.24	0.12	0.07	-10.72	0.00	0.02	Down	Yes
1.82	1.91	3.65	3.90	12.79	6.26	-1.90	0.00	0.02	Down	Yes
1.13	1.39	2.15	3.10	3.12	2.35	-1.19	0.00	0.02	Down	Yes
0.71	0.16	0.02	0.02	0.05	0.05	2.96	0.00	0.02	Up	Yes
0.41	0.59	1.07	1.15	2.00	1.64	-1.80	0.00	0.02	Down	Yes
2.04	2.34	4.56	4.89	7.69	4.74	-1.28	0.00	0.02	Down	Yes
0.92	0.28	0.25	0.12	0.08	0.18	1.83	0.00	0.02	Up	Yes
1.15	2.24	3.00	4.20	3.81	3.78	-1.04	0.00	0.02	Down	Yes
0.19	0.06	0	0	0.01	0	5.12	0.00	0.02	Up	Yes
0.45	0.14	0.03	0.05	0.03	0.05	2.32	0.00	0.02	Up	Yes
0.89	0.05	0	0.02	0	0	5.71	0.00	0.02	Up	Yes
33.20	79.68	139.90	135.72	188.88	154.33	-1.21	0.00	0.02	Down	Yes
245.36	174.71	86.07	53.01	73.36	102.17	1.03	0.00	0.02	Up	Yes
0	0	0	0.09	0.03	0.11	-9.16	0.00	0.02	Down	Yes

Supplementary File S2: Enriched gene ontology (GO) terms for the differentially expressed genes.

GO_ID	GO_Term	GO_Category	P.value	Q.value	Gene_id	Gene_name	Log2(fc)	Regulation	Significant
GO:0051482	Obsolete positive regulation of cytosolic calcium ion concentration involved in phospholipase C-activating G protein-coupled signaling pathway	Biological process	0.00	0.08	ENSGALG00010005371	GRM1	2.34	Up	Yes
GO:0051482	Obsolete positive regulation of cytosolic calcium ion concentration involved in phospholipase C-activating G protein-coupled signaling pathway	Biological process	0.00	0.08	ENSGALG00010017963	LPAR4	-1.13	Down	Yes
GO:0004375	Glycine dehydrogenase (decarboxylating) activity	Molecular function	0.01	0.08	ENSGALG00010012973	GLDC	1.34	Up	Yes
GO:0006546	Glycine catabolic process	Biological process	0.01	0.08	ENSGALG00010012973	GLDC	1.34	Up	Yes
GO:0051139	Metal cation:proton antiporter activity	Molecular function	0.01	0.08	ENSGALG00010021140	SLC11A1	-11.33	Down	Yes
GO:0015018	Galactosylgalactosylxylosylprotein 3-beta -glucuronosyltransferase activity	Molecular Function	0.01	0.08	ENSGALG00010001633	B3GAT2	1.43	Up	Yes
GO:0035672	Oligopeptide transmembrane transport	Biological process	0.01	0.08	ENSGALG00010005851	SLC15A1	1.24	Up	Yes
GO:0035673	Oligopeptide transmembrane transporter activity	Molecular function	0.01	0.08	ENSGALG00010005851	SLC15A1	1.24	Up	Yes
GO:0050254	Rhodopsin kinase activity	Molecular function	0.01	0.08	ENSGALG00010023921	GRK7	-2.17	Down	Yes
GO:0045844	Positive regulation of striated muscle tissue development	Biological process	0.01	0.08	ENSGALG00010020486	MRTFB	-1.72	Down	Yes
GO:0034123	Positive regulation of toll-like receptor signaling pathway	Biological process	0.01	0.08	ENSGALG00010017131	TLR3	-1.05	Down	Yes
GO:0034343	Type III interferon production	Biological process	0.01	0.08	ENSGALG00010017131	TLR3	-1.05	Down	Yes
GO:0034346	Positive regulation of type III interferon production	Biological process	0.01	0.08	ENSGALG00010017131	TLR3	-1.05	Down	Yes
GO:0010769	Obsolete regulation of cell morphogenesis involved in differentiation	Biological process	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0071206	Establishment of protein localization to juxtaparanode region of axon	Biological process	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0097090	Presynaptic membrane organization	Biological process	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0036146	Cellular response to mycotoxin	Biological process	0.01	0.08	ENSGALG00010028172	CERS1	-1.80	Down	Yes
GO:0072721	Cellular response to dithiothreitol	Biological process	0.01	0.08	ENSGALG00010028172	CERS1	-1.80	Down	Yes
GO:0060693	Regulation of branching involved in salivary gland morphogenesis	Biological process	0.01	0.08	ENSGALG00010016894	BTBD7	-1.22	Down	Yes
GO:0051631	Regulation of acetylcholine uptake	Biological process	0.01	0.08	ENSGALG00010011553	SLC17A8	2.73	Up	Yes
GO:0038037	G protein-coupled receptor dimeric complex	Cellular component	0.01	0.08	ENSGALG00010005371	GRM1	2.34	Up	Yes
GO:0038038	G protein-coupled receptor homodimeric complex	Cellular component	0.01	0.08	ENSGALG00010005371	GRM1	2.34	Up	Yes
GO:0098872	G protein-coupled neurotransmitter receptor activity involved in regulation of postsynaptic cytosolic calcium ion concentration	Molecular function	0.01	0.08	ENSGALG00010005371	GRM1	2.34	Up	Yes
GO:0017190	N-terminal peptidyl-aspartic acid acetylation	Biological process	0.01	0.08	ENSGALG00010027644	NAA80	-15.47	Down	Yes
GO:0018002	N-terminal peptidyl-glutamic acid acetylation	Biological process	0.01	0.08	ENSGALG00010027644	NAA80	-15.47	Down	Yes
GO:0061666	UFM1 ligase activity	Molecular function	0.01	0.08	ENSGALG00010002649	UFL1	-1.29	Down	Yes
GO:1902065	Response to L-glutamate	Biological process	0.01	0.08	ENSGALG00010002649	UFL1	-1.29	Down	Yes
GO:0140450	Protein targeting to Golgi apparatus	Biological process	0.01	0.08	ENSGALG00010013524	ZDHHC15	-1.72	Down	Yes
GO:0097309	Cap1 mRNA methylation	Biological process	0.01	0.08	ENSGALG00010020548	CMTR1	-1.34	Down	Yes

Supplementary File S2: Continue...

Supplementary File S2: Continue...

GO:0090104	Pancreatic epsilon cell differentiation	Biological process	0.01	0.08	ENSGALG00010012330	RFX6	-1.67	Down	Yes
GO:0060078	Regulation of postsynaptic membrane potential	Biological process	0.01	0.08	ENSGALG00010005371	GRM1	2.34	Up	Yes
GO:0060078	Regulation of postsynaptic membrane potential	Biological process	0.01	0.08	ENSGALG00010024157	CHRNB4	-1.75	Down	Yes
GO:0022857	Transmembrane transporter activity	Molecular function	0.01	0.08	ENSGALG00010025400	SLC22A15	-1.80	Down	Yes
GO:0022857	Transmembrane transporter activity	Molecular function	0.01	0.08	ENSGALG00010005851	SLC15A1	1.24	Up	Yes
GO:0022857	Transmembrane transporter activity	Molecular function	0.01	0.08	ENSGALG00010023918	MFSD14A	-1.32	Down	Yes
GO:1990830	Cellular response to leukemia inhibitory factor	Biological process	0.01	0.08	ENSGALG00010012973	GLDC	1.34	Up	Yes
GO:1990830	Cellular response to leukemia inhibitory factor	Biological process	0.01	0.08	ENSGALG00010021104	INA	-11.84	Down	Yes
GO:1990830	Cellular response to leukemia inhibitory factor	Biological process	0.01	0.08	ENSGALG00010016863	MYNN	-1.18	Down	Yes
GO:0007165	Signal transduction	Biological process	0.01	0.08	ENSGALG00010023921	GRK7	-2.17	Down	Yes
GO:0007165	Signal transduction	Biological process	0.01	0.08	ENSGALG00010017131	TLR3	-1.05	Down	Yes
GO:0007165	Signal transduction	Biological process	0.01	0.08	ENSGALG00010027226	OPN1MSW	2.69	Up	Yes
GO:0007165	Signal transduction	Biological process	0.01	0.08	ENSGALG00010017572	CNP3	-12.09	Down	Yes
GO:0007165	Signal transduction	Biological process	0.01	0.08	ENSGALG00010005371	GRM1	2.34	Up	Yes
GO:0007165	Signal transduction	Biological process	0.01	0.08	ENSGALG00010024157	CHRNB4	-1.75	Down	Yes
GO:0007165	Signal transduction	Biological process	0.01	0.08	ENSGALG00010017963	LPAR4	-1.13	Down	Yes
GO:0006876	Obsolete intracellular cadmium ion homeostasis	Biological process	0.01	0.08	ENSGALG00010021140	SLC11A1	-11.33	Down	Yes
GO:0046915	Transition metal ion transmembrane transporter activity	Molecular function	0.01	0.08	ENSGALG00010021140	SLC11A1	-11.33	Down	Yes
GO:0047325	Inositol tetrakisphosphate 1-kinase activity	Molecular function	0.01	0.08	ENSGALG00010015565	ITPK1	8.48	Up	Yes
GO:0052725	Inositol-1,3,4-trisphosphate 6-kinase activity	Molecular function	0.01	0.08	ENSGALG00010015565	ITPK1	8.48	Up	Yes
GO:0052726	Inositol-1,3,4-trisphosphate 5-kinase activity	Molecular function	0.01	0.08	ENSGALG00010015565	ITPK1	8.48	Up	Yes
GO:0043530	Adenosine 5'-monophosphoramidase activity	Molecular function	0.01	0.08	ENSGALG00010006344	HINT3	1.01	Up	Yes
GO:0004373	Glycogen (starch) synthase activity	Molecular function	0.01	0.08	ENSGALG00010013201	GYS2	1.53	Up	Yes
GO:0005427	Proton-dependent oligopeptide secondary active transmembrane transporter activity	Molecular function	0.01	0.08	ENSGALG00010005851	SLC15A1	1.24	Up	Yes
GO:0006857	Oligopeptide transport	Biological process	0.01	0.08	ENSGALG00010005851	SLC15A1	1.24	Up	Yes
GO:0140346	Phosphatidylserine flippase activity	Molecular function	0.01	0.08	ENSGALG00010013263	ATP11A	-1.08	Down	Yes
GO:0004530	Deoxyribonuclease I activity	Molecular function	0.01	0.08	ENSGALG00010017545	DICER1	-1.41	Down	Yes
GO:0016891	RNA endonuclease activity, producing 5′-phosphomonoesters	Molecular function	0.01	0.08	ENSGALG00010017545	DICER1	-1.41	Down	Yes
GO:0002730	Regulation of dendritic cell cytokine production	Biological process	0.01	0.08	ENSGALG00010017131	TLR3	-1.05	Down	Yes
GO:0002756	MyD88-independent toll-like receptor signaling pathway	Biological process	0.01	0.08	ENSGALG00010017131	TLR3	-1.05	Down	Yes
GO:0045163	Clustering of voltage-gated potassium channels	Biological process	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0048710	Regulation of astrocyte differentiation	Biological process	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0060168	Positive regulation of adenosine receptor signaling pathway	Biological process	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0071205	Protein localization to juxtaparanode region of axon	Biological process	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0099025	Obsolete anchored component of postsynaptic membrane	Cellular component	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0030382	Sperm mitochondrion organization	Biological process	0.01	0.08	ENSGALG00010023918	MFSD14A	-1.32	Down	Yes

Supplementary File S2: Continue...

Supplementary File S2: Continue...

GO:0051951	Positive regulation of glutamate uptake involved in transmission of nerve impulse	Biological process	0.01	0.08	ENSGALG00010011553	SLC17A8	2.73	Up	Yes
GO:0010640	Regulation of platelet-derived growth factor receptor signaling pathway	Biological process	0.01	0.08	ENSGALG00010027245	LRIG2	-1.13	Down	Yes
GO:0099530	G protein-coupled receptor activity involved in regulation of postsynaptic membrane potential	Molecular function	0.01	0.08	ENSGALG00010005371	GRM1	2.34	Up	Yes
GO:0030047	Actin modification	Biological process	0.01	0.08	ENSGALG00010027644	NAA80	-15.47	Down	Yes
GO:1905502	Acetyl-CoA binding	Molecular function	0.01	0.08	ENSGALG00010027644	NAA80	-15.47	Down	Yes
GO:0060084	Synaptic transmission involved in micturition	Biological process	0.01	0.08	ENSGALG00010024157	CHRNB4	-1.75	Down	Yes
GO:0004483	mRNA (nucleoside-2′-O-)-methyltransferase activity	Molecular function	0.01	0.08	ENSGALG00010020548	CMTR1	-1.34	Down	Yes
GO:0035898	Parathyroid hormone secretion	Biological process	0.01	0.08	ENSGALG00010021482	TRPV6	2.07	Up	Yes
GO:0003311	Pancreatic D cell differentiation	Biological process	0.01	0.08	ENSGALG00010012330	RFX6	-1.67	Down	Yes
GO:0043005	Neuron projection	Cellular component	0.01	0.08	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
GO:0043005	Neuron projection	Cellular component	0.01	0.08	ENSGALG00010005371	GRM1	2.34	Up	Yes
GO:0043005	Neuron projection	Cellular component	0.01	0.08	ENSGALG00010024157	CHRNB4	-1.75	Down	Yes
GO:0043005	Neuron projection	Cellular component	0.01	0.08	ENSGALG00010002649	UFL1	-1.29	Down	Yes
GO:1901224	Positive regulation of non-canonical NF-kappaB signal transduction	Biological process	0.01	0.09	ENSGALG00010017131	TLR3	-1.05	Down	Yes
GO:1901224	Positive regulation of non-canonical NF-kappaB signal transduction	Biological process	0.01	0.09	ENSGALG00010008274	NFAT5	-1.08	Down	Yes

the KEGG primary classification (Fig 5A). A list of all KEGG terms is provided in Supplementary File S3. The top 20 KEGG pathways with the lowest P-values were mapped. In this plot, the y-axis represents the names of the pathways and the x-axis illustrates the -log10 transformed P-values obtained from the KEGG pathway enrichment analysis (Fig 5B). The results demonstrate that, following HS, the DEGs in the resveratrol-treated group were primarily enriched in the VEGF and ErbB signaling, the insulin signaling and the Toll-like receptor signaling pathway.

Validation of DEGs by RT-qPCR

In this study, eight DEGs were selected to corroborate the gene expression profiles obtained from RNA sequencing. The eight verified DEGs included five upregulated genes (GLDC, TJP3, ACE, GYS2 and GRM1) and three down regulated genes (MLN, SRSF2 and TLR3). The RNA sequencing results were validated by RT-qPCR, demonstrating consistency between the two methods and confirming the reliability of the RNA sequencing data (Fig 6).

Chronic heat stress exposure might disturb the equilibrium between oxidative stress and antioxidant defense mechanisms. This occurs through the depletion of enzymatic antioxidants and an increase in lipid peroxidation levels (Habashy *et al.,* 2019). The poultry industry, particularly the broiler sector, is highly vulnerable to heat stress due to the birds' unique physiological structure and elevated metabolic rate. This sensitivity of broilers to HS causes oxidative imbalances, metabolic disorders and immunosuppression (Mackei *et al.,* 2021). The accumulation of ROS can lead to systemic oxidative damage, particularly affecting the intestinal mucosa and inducing muscle proteolysis (Altan *et al.,* 2003).

Resveratrol is a plant-derived polyphenolic compound that enhances immune function and exhibits antioxidant properties. It exerts multiple health benefits, including anti-inflammatory and antioxidant effects (Burns *et al.,* 2000; Putics *et al.,* 2008). There are reports indicating that resveratrol can be used as an anti-stress supplement in poultry to reduce tissue damage caused by HS (Ding *et al.,* 2023). In this research, heat-stressed chicks were given resveratrol. The results demonstrate that the addition of resveratrol for 15 days mitigated the adverse effects of HS. Specifically, the expression levels of HSPB1, HSP70 and HSP90 genes were significantly reduced when 400 mg/kg resveratrol was administered. The heat shock protein family (HSPs) are important nonspecific cell protection proteins (Hidayat *et al.,* 2020). Studies have demonstrated that animals subjected to heat stress exhibit significantly elevated mRNA expression levels of HSPs compared to those under normal conditions (Yu *et al.,* 2008; Gu *et al.,* 2012). Polyphenol supplements may play a role in regulating the expression of HSPs. The research conducted by Al-Zghoul *et al.* (2020) demonstrated that exposure to HS led to elevated levels of HSP70 expression in the intestines of pigs and rats (Al-Zghoul and Saleh, 2020). Liu *et al.* (2014) observed that under heat stress conditions,

Supplementary File S3: KEGG pathway enrichment analysis of the differentially expressed genes.

Pathway_ID	KEGG_Level_1	KEGG_Level_2	Pathway_Name	P.value	Q.value	gene_id	gene_name	log2(fc)	regulation	Significant
gga04744	Organismal systems	Sensory system	Photo transduction	0.00	0.15	ENSGALG00010023921	GRK7	-2.17	Down	Yes
gga04744	Organismal systems	Sensory system	Photo transduction	0.00	0.15	ENSGALG00010027226	OPN1MSW	2.69	Up	Yes
gga04510	Cellular processes	Cellular community -eukaryotes	Focal adhesion	0.01	0.15	ENSGALG00010027199	LAMB3	1.35	Up	Yes
gga04510	Cellular processes	Cellular community -eukaryotes	Focal adhesion	0.01	0.15	ENSGALG00010009132	SHC3	2.25	Up	Yes
gga04510	Cellular processes	Cellular community -eukaryotes	Focal adhesion	0.01	0.15	ENSGALG00010027258	ENSGALG00010027258	-1.08	Down	Yes
gga04510	Cellular processes	Cellular community -eukaryotes	Focal adhesion	0.01	0.15	ENSGALG00010018426	ENSGALG00010018426	-1.64	Down	Yes
gga00260	Metabolism	Amino acid metabolism	Glycine, serine and threonine metabolism	0.02	0.15	ENSGALG00010012973	GLDC	1.34	Up	Yes
gga00260	Metabolism	Amino acid metabolism	Glycine, serine and threonine metabolism	0.02	0.15	ENSGALG00010011814	ENSGALG00010011814	1.02	Up	Yes
gga04910	Organismal systems	Endocrine system	Insulin signaling pathway	0.02	0.15	ENSGALG00010013201	GYS2	1.53	Up	Yes
gga04910	Organismal systems	Endocrine system	Insulin signaling pathway	0.02	0.15	ENSGALG00010009132	SHC3	2.25	Up	Yes
gga04910	Organismal systems	Endocrine system	Insulin signaling pathway	0.02	0.15	ENSGALG00010027258	ENSGALG00010027258	-1.08	Down	Yes
gga04080	Environmental information processing	Signaling molecules and interaction	Neuroactive ligand-receptor interaction	0.02	0.15	ENSGALG00010027373	MLN	-2.33	Down	Yes
gga04080	Environmental information Processing	Signaling molecules and interaction	Neuroactive ligand-receptor interaction	0.02	0.15	ENSGALG00010026239	GIP	-1.21	Down	Yes
gga04080	Environmental information processing	Signaling molecules and interaction	Neuroactive ligand-receptor interaction	0.02	0.15	ENSGALG00010005371	GRM1	2.34	Up	Yes
gga04080	Environmental information processing	Signaling molecules and interaction	Neuroactive ligand-receptor interaction	0.02	0.15	ENSGALG00010024157	CHRNB4	-1.75	Down	Yes
gga04080	Environmental information processing	Signaling molecules and interaction	Neuroactive ligand-receptor interaction	0.02	0.15	ENSGALG00010017963	LPAR4	-1.13	Down	Yes
gga04012	Environmental information processing	Signal transduction	ErbB signaling pathway	0.06	0.32	ENSGALG00010009132	SHC3	2.25	Up	Yes
gga04012	Environmental information processing	Signal transduction	ErbB signaling pathway	0.06	0.32	ENSGALG00010027258	ENSGALG00010027258	-1.08	Down	Yes
gga04512	Environmental information processing	Signaling molecules and interaction	ECM-receptor interaction	0.06	0.32	ENSGALG00010027199	LAMB3	1.35	Up	Yes
gga04512	Environmental information processing	Signaling molecules and interaction	ECM-receptor interaction	0.06	0.32	ENSGALG00010018426	ENSGALG00010018426	-1.64	Down	Yes
gga00500	Metabolism	Carbohydrate metabolism	Starch and sucrose metabolism	0.14	0.58	ENSGALG00010013201	GYS2	1.53	Up	Yes

Supplementary File S3: Continue...

Supplementary File S3: Continue...

gga00630	Metabolism	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	0.15	0.58	ENSGALG00010012973	GLDC	1.34	Up	Yes
gga05168	Human diseases	Infectious disease: Viral	Herpes simplex virus 1 infection	0.16	0.58	ENSGALG00010029370	SRSF2	-1.58	Down	Yes
gga05168	Human diseases	Infectious disease: Viral	Herpes simplex virus 1 infection	0.16	0.58	ENSGALG00010017131	TLR3	-1.05	Down	Yes
gga00270	Metabolism	Amino acid metabolism	Cysteine and methionine metabolism	0.20	0.60	ENSGALG00010011814	ENSGALG00010011814	1.02	Up	Yes
				0.22	0.60	ENSGALG00010028172	CERS1	-1.80	Down	Yes
gga00600	Metabolism	Lipid metabolism	Sphingolipid metabolism							
gga04370	Environmental information processing	Signal transduction	VEGF signaling pathway	0.26	0.60	ENSGALG00010027258	ENSGALG00010027258	-1.08	Down	Yes
gga01230	Metabolism	Global and overview maps	Biosynthesis of amino acids	0.26	0.60	ENSGALG00010011814	ENSGALG00010011814	1.02	Up	Yes
gga00562	Metabolism	Carbohydrate metabolism	Inositol phosphate metabolism	0.30	0.60	ENSGALG00010015565	ITPK1	8.48	Up	Yes
gga04144	Cellular processes	Transport and catabolism	Endocytosis	0.32	0.60	ENSGALG00010023921	GRK7	-2.17	Down	Yes
gga04144	Cellular processes	Transport and catabolism	Endocytosis	0.32	0.60	ENSGALG00010027975	IQSEC1	-1.47	Down	Yes
gga04914	Organismal systems	Endocrine system	Progesterone-mediated oocyte maturation	0.33	0.60	ENSGALG00010015487	RPS6KA6	-1.25	Down	Yes
gga04540	Cellular processes	Cellular community -eukaryotes	Gap junction	0.34	0.60	ENSGALG00010005371	GRM1	2.34	Up	Yes
gga04620	Organismal systems	Immune system	Toll-like receptor signaling pathway	0.35	0.60	ENSGALG00010017131	TLR3	-1.05	Down	Yes
gga04070	Environmental information processing	Signal transduction	Phosphatidylinositol signaling system	0.37	0.60	ENSGALG00010015565	ITPK1	8.48	Up	Yes
gga04114	Cellular processes	Cell growth and death	Oocyte meiosis	0.39	0.60	ENSGALG00010015487	RPS6KA6	-1.25	Down	Yes
gga01200	Metabolism	Global and overview maps	Carbon metabolism	0.41	0.60	ENSGALG00010012973	GLDC	1.34	Up	Yes
gga04514	Environmental information processing	Signaling molecules and interaction	Cell adhesion molecules	0.44	0.60	ENSGALG00010027385	CNTN2	-4.99	Down	Yes
gga04270	Organismal systems	Circulatory system	Vascular smooth muscle contraction	0.45	0.60	ENSGALG00010017572	CNP3	-12.09	Down	Yes
gga04068	Environmental information processing	Signal transduction	FoxO signaling pathway	0.46	0.60	ENSGALG00010005371	GRM1	2.34	Up	Yes
gga04142	Cellular processes	Transport and catabolism	Lysosome	0.46	0.60	ENSGALG00010021140	SLC11A1	-11.33	Down	Yes
gga04217	Cellular processes	Cell growth and death	Necroptosis	0.47	0.60	ENSGALG00010017131	TLR3	-1.05	Down	Yes
gga03040	Genetic information processing	Transcription	Spliceosome	0.50	0.60	ENSGALG00010029370	SRSF2	-1.58	Down	Yes

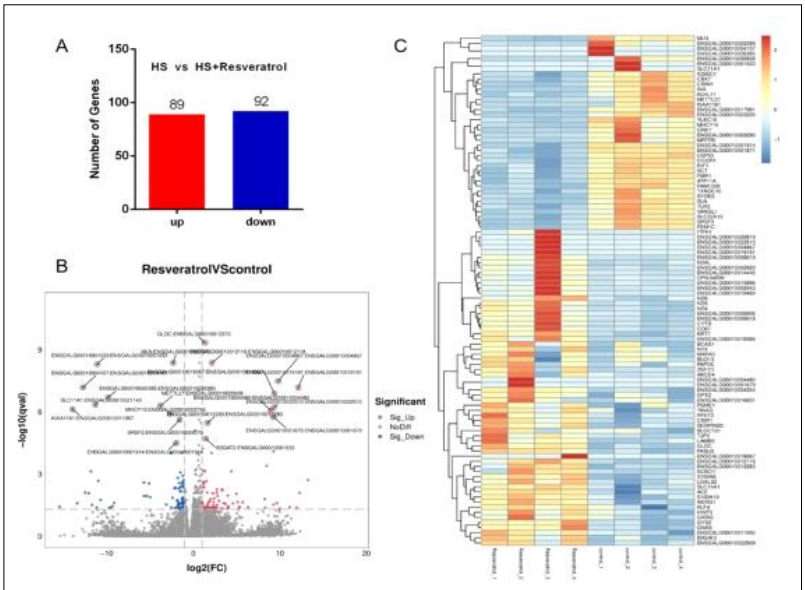
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gga05164	Human diseases	Infectious disease: Viral	Influenza A	0.50	0.60	ENSGALG00010017131	TLR3	-1.05	Down	Yes
gga04310	Environmental information processing	Signal transduction	Wnt signaling pathway	0.51	0.60	ENSGALG00010025428	VANGL1	-1.55	Down	Yes
gga04150	Environmental information processing	Signal transduction	mTOR signaling pathway	0.52	0.60	ENSGALG00010015487	RPS6KA6	-1.25	Down	Yes
gga04530	Cellular processes	Cellular community -eukaryotes	Tight junction	0.53	0.60	ENSGALG00010028109	TJP3	1.07	Up	Yes
gga04810	Cellular processes	Cell motility	Regulation of actin cytoskeleton	0.63	0.69	ENSGALG00010017963	LPAR4	-1.13	Down	Yes
gga04020	Environmental information processing	Signal transduction	Calcium signaling pathway	0.68	0.72	ENSGALG00010005371	GRM1	2.34	Up	Yes
gga04010	Environmental information processing	Signal transduction	MAPK signaling pathway	0.73	0.75	ENSGALG00010015487	RPS6KA6	-1.25	Down	Yes
gga01100	Metabolism	Global and overview maps	Metabolic pathways	0.83	0.83	ENSGALG00010012973	GLDC	1.34	Up	Yes
gga01100	Metabolism	Global and overview maps	Metabolic pathways	0.83	0.83	ENSGALG00010015565	ITPK1	8.48	Up	Yes
gga01100	Metabolism	Global and overview maps	Metabolic pathways	0.83	0.83	ENSGALG00010013201	GYS2	1.53	Up	Yes
gga01100	Metabolism	Global and overview maps	Metabolic pathways	0.83	0.83	ENSGALG00010028172	CERS1	-1.80	Down	Yes
gga01100	Metabolism	Global and overview maps	Metabolic pathways	0.83	0.83	ENSGALG00010011814	ENSGALG00010011814	1.02	Up	Yes

the expression levels of HSP70 and HSP90 in the jejunum tissue of Muchuan black bone Chicken were upregulated (Liu *et al.,* 2014). These findings align closely with the outcomes of our current study on the intestinal mucosa of chickens under similar conditions.

Excessive reactive oxygen species generated under heat stress can initiate lipid peroxidation reactions, leading to oxidative damage and inflammatory responses in cellular proteins and DNA, ultimately compromising the integrity of intestinal tissues. When cellular oxidative status is disrupted, the activity of key antioxidants such as T-AOC, POD, SOD, CAT, TPX and GSH-Px decreases, while MDA is overproduced (Circu and Aw, 2010; Yang *et al.,* 2010). It has been demonstrated that resveratrol effectively prevents the formation of free radicals induced by HS, lipopolysaccharide stress and ultraviolet stress, while also inactivating oxidative precursors generated during these stress conditions. Studies have indicated that supplementing the diet with resveratrol can significantly alle*via*te HS-induced body damage in rats (Das, 2011) and quail (Sahin *et al.,* 2012), while boosting the activities of SOD, CAT and GSH-PX and lowering MDA levels. According to Liu *et al.* (2014), the inclusion of 400 mg/kg resveratrol in the diet led to a notable enhancement in the serum GPX,



(A) The numbers of upregulated and downregulated DEGs. (B) Volcano plot showing the DEGs. (C) Heat map of DEG clustering for each sample.

Fig 3: RNA sequencing analysis.

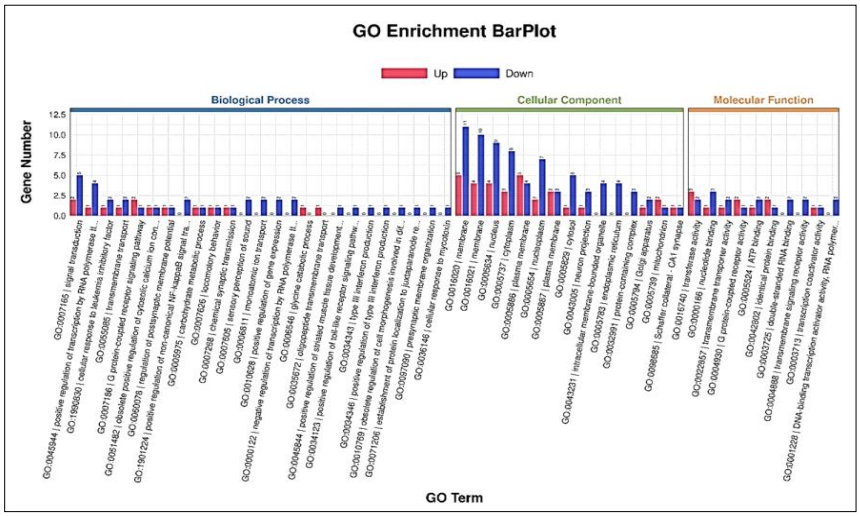


Fig 4: GO analysis of the DEGs.

SOD and CAT enzyme activities in black-boned chickens on the fifteenth day of exposure to heat stress. Additionally, it increased GSH content while reducing MDA levels (Liu *et al.,* 2014). This study was conducted under conditions where chicks were exposed to prolonged HS. Compared with the normal temperature control group, heat-stressed chicks exhibited significantly reduced serum activities of T-AOC, SOD, CAT, POD and GSH-Px, along with an increased level of MDA. However, under heat stress conditions, supplementation with resveratrol led to a significant recovery in these antioxidant indices and a marked decrease in MDA levels. These findings suggest that resveratrol can effectively mitigate oxidative damage in the body under HS conditions.

Heat stress can induce systemic responses in chicks. Maintaining the integrity of intestinal structure and function, which is vital for nutrient absorption, is essential for poultry health (Liu *et al.,* 2022). Burkholder *et al.* (2008) found that HS can compromise the structure of intestinal villi and disrupt the normal microbiota in poultry (Burkholder *et al.,* 2008). Quinteiro *et al.* (2012) also demonstrated that HS is capable of causing intestinal damage and triggering acute enteritis in poultry (Quinteiro *et al.,* 2012). The pathological damage induced by HS mainly involves the mucosal layer,

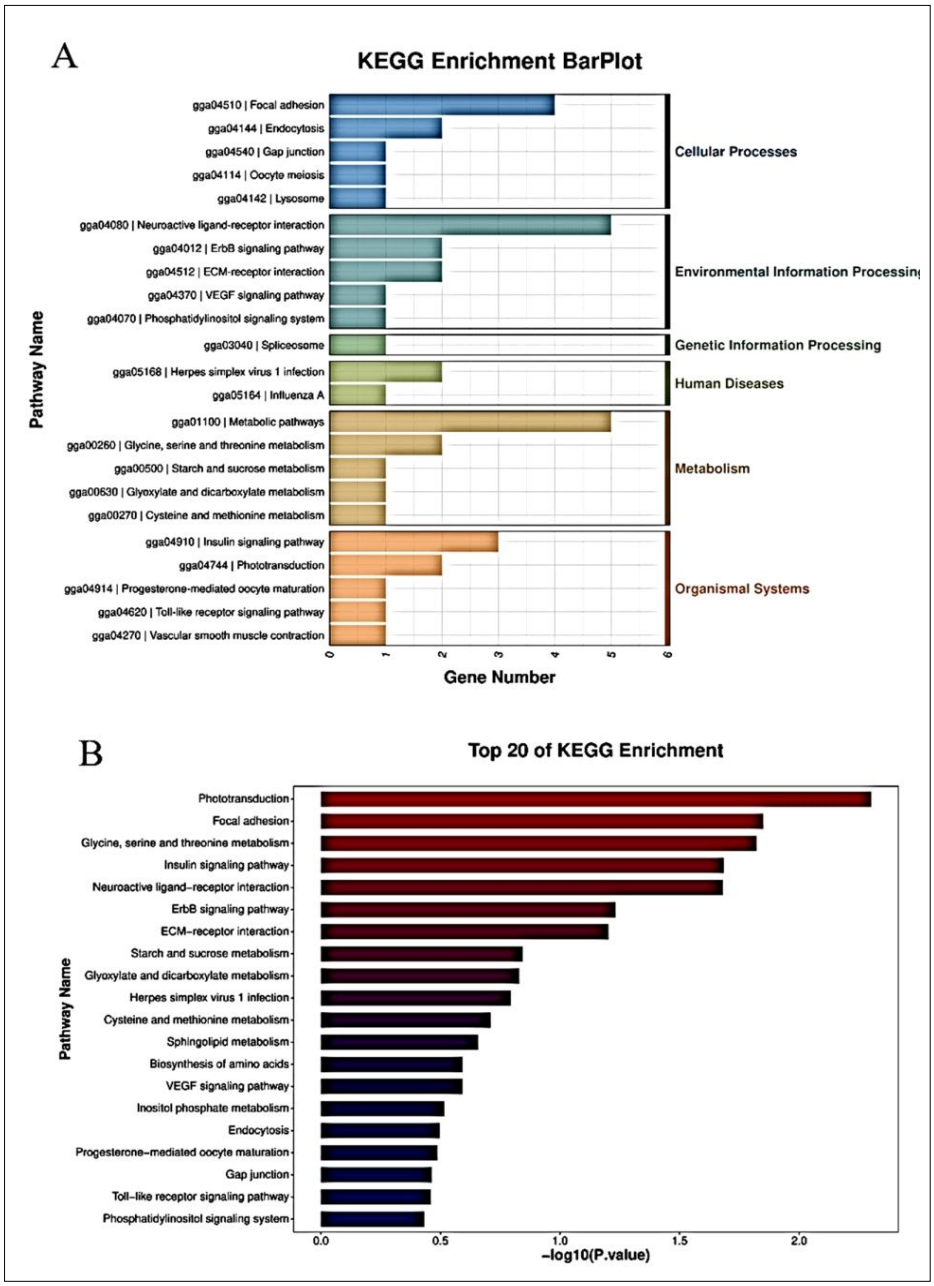
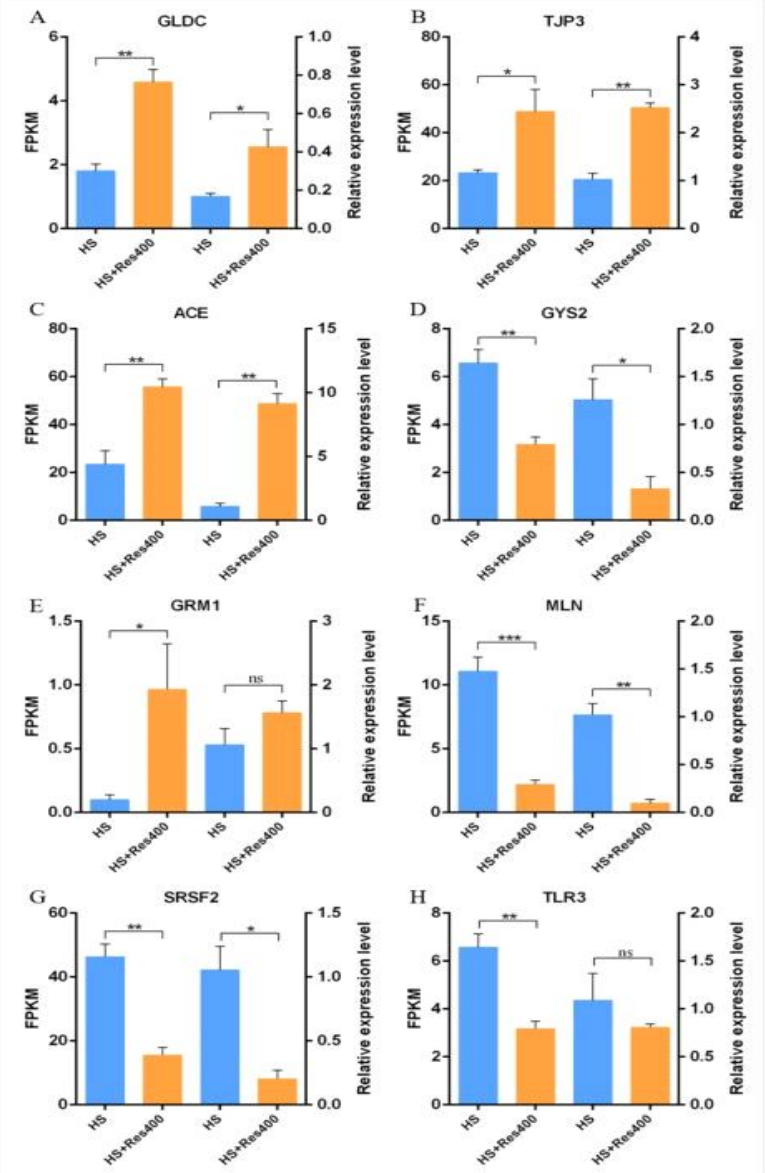


Fig 5: KEGG enrichment analysis of DEGs. (A) KEGG enrichment. (B) Top 20 significantly enriched KEGG pathways.

manifesting as exfoliation of mucosal epithelial cells and disruption of intestinal villi (Rostagno, 2020). This finding aligns with the outcomes of the current research. Resveratrol was found to markedly enhance the villus height in the duodenum and jejunum, effectively reducing the intestinal mucosal structural damage induced by HS.

The regulation of the heat stress response in poultry is a complex process involving multiple genes. To explore the protective mechanism of resveratrol on the gut of chicks under HS, we performed transcriptome sequencing of the jejunal mucosa of chicks fed with resveratrol. Through high-throughput sequencing technology, 181 DEGs were identified, of which 89 were significantly upregulated, including SHC3, GLDC, CKB, GYS2, GRM1, ITPK1, TJP3, LAMB3, ACE, NOXO1 and ND6. In addition, 92 were significantly downregulated, including MHCY15, SRSF2, INA, USP53, ATP11A, MRTFB, CNP3, CNTN2 and GIP. The DEGs were involved in various biological pathways, including developmental processes, response to multicellular stimuli, oxidative homeostasis, neural cell differentiation and development, phosphotransferase activity, nucleic acid binding and transcriptional regulation, as well as immune and extracellular domain responses. These results indicate that resveratrol induced alterations in multiple genes in the chick gut.



Data are expressed as means ± SEM (n =4). The one-way ANOVA was used for statistical significance. (ns: *P*> 0.05;* *P* <0.05; ** *P* <0.01).

Fig 6: Confirmation of the transcriptome sequencing data by RT-qPCR.

The results of GO annotation and KEGG pathway analysis show that, in comparison to the blank control group, the DEGs were mainly enriched in pathways including VEGF signaling, ErbB, insulin signaling and Toll-like receptor signaling. The VEGF signaling pathway is alternatively referred to as the vascular endothelial growth factor signaling pathway. The signaling initiated by VEGF/VEGFR2 can cause endothelial cell proliferation and increase vascular permeability, leading to vascular tumors, edema, inflammation and vascular dysfunction (Yang and Cao, 2022, (Apte *et al.,* 2019). This pathway may be related to intestinal inflammation under HS (Scaldaferri *et al.,* 2009). Our sequencing results revealed that genes associated with this pathway were markedly downregulated in the resveratrol-treated group, providing evidence for positive effects of resveratrol on HS-induced intestinal inflammation. The ErbB pathway exerts control over cell proliferation, differentiation and migration through its interaction with several key signaling cascades, including the PI3K/Akt, JAK/STAT and MAPK pathways (Grant *et al.,* 2002). Depending on the specific cellular context, ErbB receptor activation may promote proliferation, motile adhesion, differentiation or even apoptosis (Appert-Collin *et al.,* 2015). The insulin signaling pathway is a biochemical chain of reactions used to transmit information about the insulin hormone to the interior of the cell to regulate metabolic activity and growth (Hotamisligil and Davis, 2016). The insulin signaling pathway is essential for controlling blood glucose levels, fat metabolism and protein synthesis (Saltiel, 2021). Our sequencing data show that genes enriched in this pathway, such as GYS2 and SHC3, were significantly upregulated in the resveratrol-treated group. This indicates that resveratrol can participate in regulating glycogen metabolism under heat stress conditions.

In general, the physiological functions and mechanisms of resveratrol are many, including oxidative stress protection, immune system regulation, growth and development support and cell signal transduction regulation. Together, these effects contribute positively to the development, intestinal health and immune system of heat-stressed chicks. However, the specific mechanisms and effects of resveratrol still require further elucidation. Future studies need to clarify its precise pathway of action in chicks as well as determine the most suitable dosage, in order to better harness the potential of resveratrol in animal husbandry.

CONCLUSION

Heat stress can lead to intestinal epithelial damage and oxidative stress in broilers. Dietary supplementation with 400 mg/kg resveratrol can effectively mitigate the adverse effects of heat stress. Transcriptomic analyses indicate that the protective mechanism of resveratrol involves modulating genes associated with key signaling pathways. In conclusion, resveratrol may serve as an effective nutritional modifier for alleviating heat stress in poultry.

ACKNOWLEDGEMENT

Not applicable.

Funding

This work was supported by the Natural Science Foundation of Sichuan Province of China [Grant No. 2023NSFSC0231] and the Southern Sichuan local breed chicken Research Center of Sichuan University project [Grant No. 2025DFPZJ002]. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Data availability statement

The data that support this study are available in the article and accompanying online supplementary material.

Disclaimers

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Informed consent

All experiments involving animals and study protocols were approved by the Animal Care and Use Commission of the College of Life science, Leshan Normal University.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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