Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2023 / v.25 / n.4 / 001-012

http://dx.doi.org/10.1590/1806-9061-2023-1825

Original Article

Effects of Heat Stress on Production Indices, Antioxidant Function, Heat Shock Protein and Intestinal Microflora in Quails

■Author(s)

Qin Q[I] https://orcid.org/0000-0001-5951-5920
Ma B[I] https://orcid.org/0009-0002-4299-5862
Li Z[I] https://orcid.org/0000-0002-1684-0317
Wu H[I] https://orcid.org/0000-0002-8565-960X
Zhu J[I] https://orcid.org/0009-0007-6800-0604
Li X[I] https://orcid.org/0009-0008-8327-7166
Wang K[I] https://orcid.org/0009-0004-7127-935X
Yi X[I] https://orcid.org/0000-0002-7959-116X

[I] College of Animal Science and Technology, Xinyang Agriculture and Forestry University, Xinyang, Henan Province, P. R. China.

■Mail Address

Corresponding author e-mail address
Qingming Qin
Xinyang Agriculture and Forestry University, Xinyang, Henan, 464000, China.
Phone: +86 15137304502
Email: qinqingming@xyafu.edu.cn

■Keywords

Antioxidant function, Heat shock protein, Heat stress, Intestinal microbiota, Quail.

Submitted: 30/June/2023
Approved: 08/October/2023

ABSTRACT

Gut microbiota plays an important role in animal health, production and diseases. Little is known about whether heat stress alters the composition of quail gut microbiota; therefore, we studied the effects of heat stress on growth performance, antioxidant functions, heat shock proteins and caecal microbiota. Two groups of 40 (20-day-old) quails were set up, including a control reared at 24 ± 2°C and a heat stress group subjected to heat stress at 36 ± 2°C for 4 h per day for 7 consecutive days. We found that heat stress significantly elevated the relative expression levels of HSP70 and HSP90 mRNA in the thymus, bursa and spleen by real-time fluorescence quantitative PCR assay, indicating successful establishment of the experimental model. Heat stress was found to have an effect on gut microbiota composition. At the genus level, *Alistipes* were significantly increased in the heat stress group. PICRUSt2 function prediction revealed that most of the KEGG pathways with high temperature-induced abundance differences are metabolism-related. These data indicated that heat stress reduced the production performance of quails by affecting antioxidant functions, as well as the composition and structure of the intestinal microbiota. The results of this study provide technical information for conducting research on heat stress prevention and control techniques in quails.

INTRODUCTION

The livestock industry and especially poultry farming are facing the challenge of global warming. Poultrys' physiological and performance characteristics can be adversely affected by high environmental temperatures (El-Kholy *et al*., 2017). High body temperatures lead to high mortality and growth inhibition in poultry, resulting in huge economic losses (Bartlett & Smith, 2003). As a result of oxidative stress, animals experience a negative immune response and health status (Sordillo & Aitken, 2009). Moreover, heat stress can result in oxidative stress in poultry, increasing their pathogen susceptibility (Shakeri *et al*., 2019). Therefore, determining the effects that thermal stress has on poultry can assist in developing novel strategies to protect poultry under heat stress.

Heat shock proteins (HSP) can recognize, restore or degrade misfolded proteins induced by high temperature, reactive oxygen species and other stress states in animal cells (Vabulas *et al*., 2010). In particular, HSP70 and HSP90 are ubiquitous in eukaryotes and prokaryotes and are highly conserved molecular chaperones (Lang *et al*., 2022; Gupta *et al*., 2020). Under heat stress, poultry respond by significant increases in HSP70 and/or HSP90 levels in organs such as the brain, spleen, heart, liver, bursa of Fabricius and in chicken muscles (Liu *et al*., 2014; Xie *et al*., 2014; Han *et al*., 2019). In quails, similar results were found for the liver (Sahin *et al*., 2012; Mehaisen *et al*., 2017) and serum, brain

and ovary (Sahin *et al*., 2009). However, there need to be further studies documenting heat stress impacts on HSP70 and HSP90 levels in immune organs in poultry.

There is increasing evidence that intestinal microorganisms play pivotal roles in animal health and production by regulating energy homeostasis, intestinal epithelium health, immunocompetence, and disease tolerance (Smith *et al*., 2013; Oakley *et al*., 2014; Barko *et al*., 2018). It is also possible for ambient temperature, diet and diseases to alter the composition of the gut microbiota (David *et al*., 2014; Mckenzie *et al*., 2017; Fontaine *et al*., 2018). These stressors can destroy the symbiotic relationships in the host, leading to intestinal microbiota disorder and increasing disease susceptibility (Lewis *et al*., 2015; Gur & Bailey, 2016). There are a variety of microbes in the gastrointestinal tracts of poultry that have been linked to health and disease protection. For instance, 16S sequencing techniques have confirmed that heat stress alters the intestinal microbiota of ducks (Tian *et al*., 2020), broilers (Liu *et al*., 2020) and laying hens (Zhu *et al*., 2019), but there is no data for quails. However, we speculate that heat stress may disrupt the caecal microbiota of quails, reduce their diversity, and reduce antioxidant capacity, thus affecting their production performance.

Quail meat and eggs can provide high quality protein with low calories and high biological value (Mehaisen *et al*., 2019). Moreover, compared with other poultry species, this bird is also an ideal laboratory animal (Eldaly *et al*., 2013). In this study, high-throughput sequencing techniques were used to investigate the effects of high temperature on the intestinal microbiota of quails to explore the relationship between heat stress and the intestinal microbiota. We used these data to analyze correlations between abundant microbiota, production performance, antioxidant indices and heat shock protein levels to provide evidence for remission of the adverse reactions of hyperthermia by regulating intestinal microbiota.

MATERIALS AND METHODS

Experimental design, and diets

Quails were provided by a farm in Henan Province and fed adaptively for 10 days. The heat stress test began at 20 days of age. We randomly divided quails (n=80) with similar body weights into two groups: control (W) and heat stress (WH) group. A total of 4 replicas were set up for each group, each with 10 quails. The ambient temperature of group W was kept at 24 ± 2°C, with a relative humidity of 55 ± 2%. For 7 consecutive days, group WH was subjected to heat stress every day for 4 h from 12:00 to 16:00 at 36 ± 2°C and humidity of 70 ± 2, and the remainder of the time were kept in the same conditions of group W. The birds were fed basic diet (corn-soybean meal diet) (Table 1) and randomly received feed and drinking water.

Table 1 – Dietary composition and nutrient levels.

Ingredient	Content (%)	Nutrient composition	Content (%)
Maize	51.09	Metabolizable energy / (MJ/kg)	11.92
Soybean meal	35.00	Crude protein/%	23.76
Soya-bean oil	1.50	Crude fibre/%	3.47
Fish meal	9.00	Coarse ash/%	6.54
$CaHPO_4$	0.70	Available phosphorus/%	0.32
$CaCO_3$	1.25	Calcium/%	0.82
Sodium chloride	0.30		
DL-Methionine	0.13		
Lysine	0.03		
Premix [1]	1.00		
Total	100		

[1] Premix provided the following per kilogram of diet: vitamin A, 12000 IU; vitamin D_3, 5000 IU; vitamin K_3, 2 mg; vitamin E, 2500 IU, vitamin B_1, 200 mg; vitamin B_2, 350 mg; vitamin B_6, 300 mg; vitamin B_{12}, 1 mg; niacin, 3 mg; pantothenic acid, 800 mg; folic acid, 500 mg; biotin, 0.2 mg; choline chloride, 1 mg; Fe, 10 mg; Cu 12 mg; Mn, 30 mg; I, 30 mg; Se 20 mg.

Production performance

Initial and daily weights, as well as feed consumption, were recorded. On day 8, the birds were weighed on an empty stomach. The results were used for calculations of the average daily feed intake (ADFI), average daily gain (ADG), and feed / gain ratio (F/G).

Detection of serum antioxidant indices

After the experiment ended, one quail from each replicate group was selected to be blood sampled from the fasted right jugular vein. By centrifuging the serum for 10 minutes at 3500 rpm, the serum was separated. The antioxidant indexes for glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and malondialdehyde (MDA) were determined using ELISA kits (Sino Best Biological Technology, Shanghai, China) following the protocol provided by the manufacturer.

Heat shock protein expression

HSP mRNA level was quantified in bursa of Fabricius, thymus and spleen tissues of quails sacrificed by cervical dislocation. Total RNA was extracted using a commercial kit (Wuhan Servicebio Technology, Wuhan, China) and quantified through a UV spectroscopy instrument (NanoDrop 2000, Thermo Fisher, Pittsburg, PA, USA). HSP70 and HSP90 mRNA expression ratios were calculated by fluorescence quantitative RT-PCR (qPCR) methods using quail-specific PCR primers

Table 2 – Primer pairs for qPCR.

Target	Accession number	Primer sequence	PCR product size (bp)
HSP70	XM_015876197.2	F: 5′-ATGAAACTGAGTCGCTCGCA-3′	166
		R: 5′-CAGTCTGTTGCACCTTTGGC-3′	
HSP90	NM_001323197.1	F: 5′-GAAACACTCTGGGACGTGGT-3′	158
		R: 5′-TTCGACAGTCTCCGTCTTGC-3′	
β-actin	NM_001124235.1	F: 5′-CTGGCACCTAGCACAATGAA-3′	123
		R: 5′-CTGCTTGCTGATCCACATCT-3′	

(Table 2), which were synthesized by GENEWIZ, Suzhou, China. Reverse transcription reactions were performed with a commercial kit (Wuhan Servicebio) using the manufacturers' protocol, and cDNA was stored at -20°C. The specific steps and procedures of qPCR were completed following Li *et al*. (2023). We calculated the expression of mRNAs using the $2^{-\triangle\triangle CT}$ method, through the software supplied with the RT-PCR instrument.

Bioinformatic analysis of intestinal microbiota

Samples of quail caecal contents were collected and frozen in liquid nitrogen. Samples were identified using 16S rDNA detection completed by Shanghai Origin gene Bio-pharm Technology using the V3-V4 region. The primer sequence was as follows: 338F: 5′-ACTCCTACGGGAGGCAGCAG-3′, 806R: 5-GGACTACHVGGGTWTCTAAT-3′. Illumina PE250 sequencing was used to generate the caecal library. The raw data was processed using the QIIME software (Caporaso *et al*., 2010). Operational taxonomic units (OTUs) were merged and classified based on a 97 % sequence identity, using the UCLUST sequence alignment tool (Edgar, 2010). Species annotations were carried out using the Ribosomal Database Project database, and α and β diversity were analyzed using QIIME. Differences in species classification of intestinal microbiota were analyzed using the LEfSe method (Segata *et al*., 2011). R version 2.15 was used to display the data. The functions were predicted and analyzed with PICRUSt2 (Douglas *et al*., 2019).

Statistical analysis

An analysis of production and antioxidant indices was conducted using Prism 8 (GraphPad, La Jolla, CA, USA) with Student's *t*-test. Heat shock proteins were analyzed and graphed using R v.3.6.3 (https://www.xiantao.love/products). STAMP 2.1.3 was used for the Welch *t*-test at genus level, function prediction, and chart construction. We presented the results as means + standard error of the mean.

RESULTS

Heat stress affects HSP expression in quail immune organs

Heat-stressed animals had significantly higher *HSP70* and *HSP90* levels in the thymus and bursa of Fabricius ($p<0.01$). *HSP70* mRNA in spleens of thermal-stressed quails also significantly increased ($p<0.01$) (Figure 1).

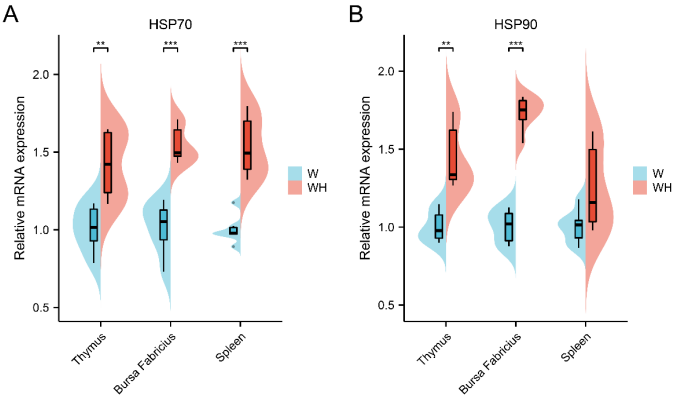

Figure 1 – Effect of heat stress on the relative expressions of HSP70 and HSP90 mRNA in the immune organs of quails. (A) *HSP70*, (B) *HSP90*. W: control group; WH: heat stress group. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Heat stress reduces quail production performance

Thermal stress in quails significantly decreased ($p<0.01$) ADG, significantly increased ($p<0.05$) F/G, and decreased ($p>0.05$) ADFI by 9.73% as compared with the W group (Table 3).

Table 3 – Effect of heat stress on production performance of quails.

Item	W	WH	*p*-value
ADFI (g)	18.92±0.80	17.08±1.25	0.2618
ADG (g)	4.76±0.18^{A}	3.75±0.12^{B}	0.0034
F/G	3.98±0.05^{a}	4.54±0.21^{b}	0.0389

The means and standard error of the means are used to express data.

$^{A, B \text{ and } a,b}$ Different uppercase and lowercase letters on each value showed significant differences ($p<0.01$, $p<0.05$, respectively). W: control group; WH: heat stress group.

Heat stress inhibits the antioxidant function of quail serum

The activity of SOD and GSH-Px for the animals in a heat stress environment were significantly (p=0.0475 and p=0.0013, respectively) decreased. A heat-stressed quail's MDA level was significantly higher (p=0.0134) (Table 4).

Table 4 – Effect of heat stress on serum antioxidant indices in experimental quails.

Item	W	WH	p-value
SOD (ng/mL)	11.80±0.34^{a}	10.82±0.19^{b}	0.0475
GSH-Px (ng/mL)	199.06±3.49^{A}	170.92±3.52^{B}	0.0013
MDA (nmol/mL)	16.54±0.77^{b}	19.27±0.15^{a}	0.0134

See Table 3 for abbreviations.

Heat stress affects the diversity of caecal microbiota in quails

The α diversity analysis of the intestinal microbiota of our experimental quails indicated that the diversity of group WH was significantly lower when compared to group W (Ace index, Chao index p<0.01; Shannon index p<0.05) (Figure 2A, 2B and 2C). The numbers of unique OTUs in group W was 436 and decreased to 188 in group WH (Figure 2D). Principal component analysis (PCA) also demonstrated a significant difference between groups W and WH (Figure 2E). Cluster analysis of species composition among samples revealed that W and WH were contained within different branches, indicating a low similarity of species compositions (Figure 2F).

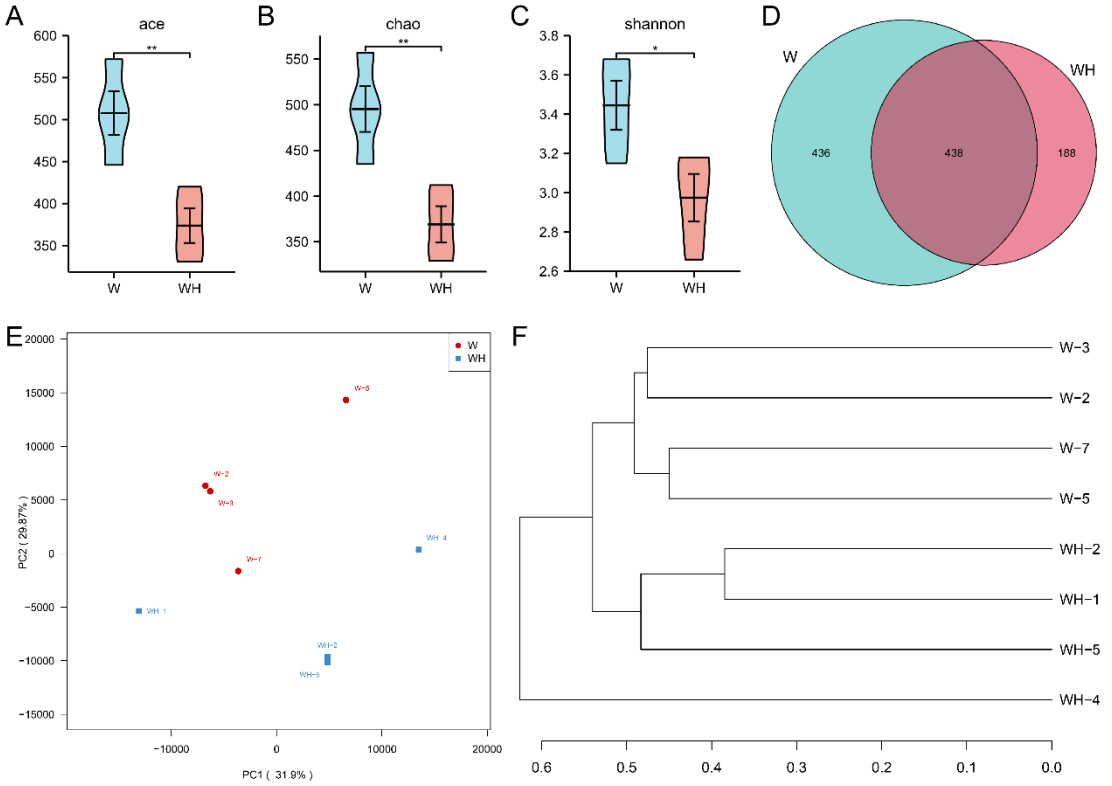

Figure 2 – Effects of heat stress on the diversity of caecal microbiota in quails. (A-C) Ace, Chao and Shannon index. (D) Venn diagram. (E) A principal component analysis (PCA) model based on operational taxonomic units (OTUs) was used to analyze the β-diversity of microbial communities. Percentages represent the contribution of principal components to sample differences. (F) Sample evolutionary tree analysis diagram. Cluster Tree Analysis based on UniFrac distances. W: control group; WH: heat stress group. *, p<0.05; **, p<0.01.

Analysis of the difference in abundance of intestinal microbiota

The dominant caecal microbiota phylum-wise (W versus WH) increased for *Bacteroidetes* (44.95% versus 52.35) and *Verrucomicrobia* (0 versus 6.14 %), and decreased for *Firmicutes* (35 versus 28.01 %), *Actinobacteria* (16.58 versus 12.64 %), *Proteobacteria* (1.18 versus 0.40%) and *Saccharibacteria* (0.86 versus 0.07 %) (Figure 3A, 3C and 3D). There were 163 genera identified in the caecal contents of the two groups at the genus level. *Alistipes* was the dominant genus for WH and its abundance was significantly (p=0.011) greater than in group W (Figure 3B and 3E).

LEfSe analysis of caecal microbiota

The linear discriminant analysis effect size (LEfSe) can identify biomarkers that significantly differ

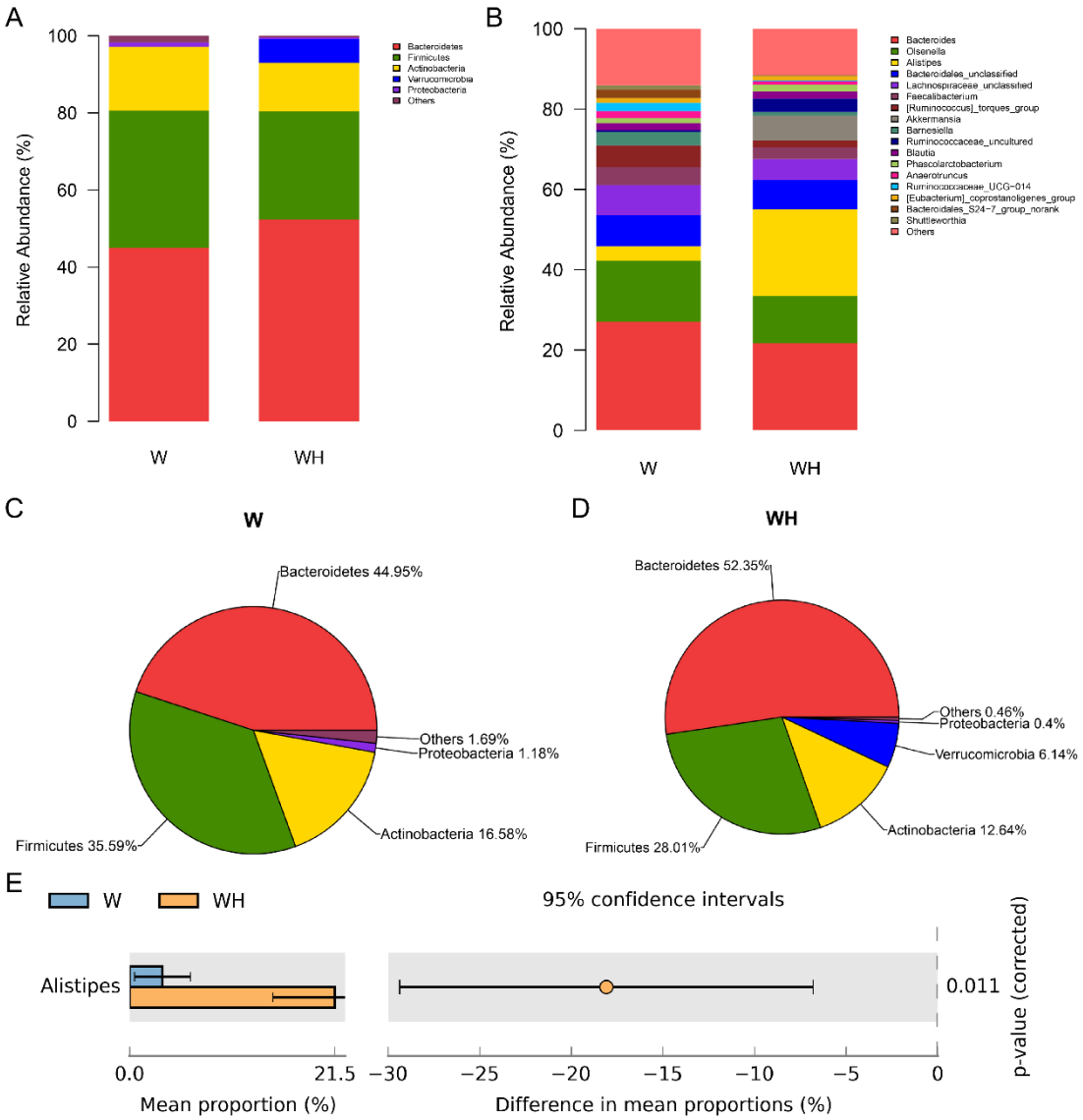

Figure 3 – An investigation of the effects of high temperatures on the caecal microbiota of quails at different taxonomic levels. (A) Distributions at the phylum taxonomic level. (B) Distributions at the genus taxonomic level. Percentage of microbial composition at phylum taxonomic level in (C) group W and (D) group WH. (E) Analysis of differences in caecal microbial composition at the genus taxonomic level. W: control group; WH: heat stress group.

statistically between groups. LDA value distributions for each group of samples identified 19 genera that displayed significant differences between groups W and WH. In group WH, the genera that were more present were *Akkermansia, Alistipes, Anaerofustis, Butyricimonas, Coriobacteriaceae_g_uncultured, Eubacterium, Flavobacteriaceae_g_uncultured,* and *Streptococcus* (Figure 4).

Functional prediction of caecal microbiota

We used PICRUSt2 to predict functions for the significant microbiota we identified. Functions for DDT degradation, fatty acid biosynthesis, and tetracycline biosynthesis in group WH were significantly less than those of group W ($p<0.05$). Annotations for taurine and hypotaurine metabolism, renin-angiotensin system, streptomycin biosynthesis, apoptosis, stilbenoid, diarylheptanoid and gingerol biosynthesis in WH were significantly higher than those in group W ($p<0.05$) (Figure 5).

Correlations between caecal microbes and healthy parameters

We additionally applied a Spearman correlation analysis to further reveal potential correlations between intestinal microbiota, immune organ *HSP70* and *HSP90* gene expression levels, serum antioxidant levels, and production performance indices. At the phylum level, we found a significant positive correlation between *Bacteroidetes* and ADG, and a significant negative

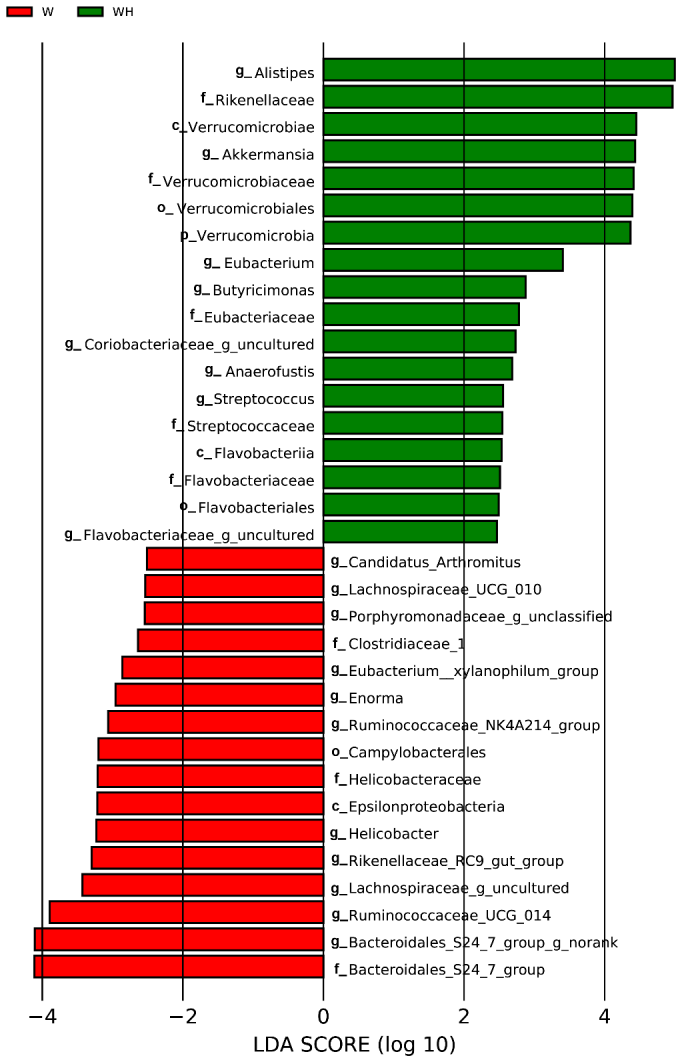

Figure 4 – Linear discriminant analysis (LDA) of caecal microbiota in quails. LDA score is represented by the length of the histogram. Abscissa, species LDA score. LDA > 2 represents statistically significant biomarkers. W: control group; WH: heat stress group.

correlation between *Bacteroidetes* and MDA among caecal microorganisms ($p<0.05$). *Firmicutes* was positively correlated with the relative expression of *HSP70* in spleen, *HSP90* in thymus and *HSP90* mRNA in bursa of Fabricius, as well as with SOD activity ($p<0.05$) (Figure 6A). At the genus level, LEfSe analysis indicated that a significantly strong presence of *Streptococcus* was positively correlated with the relative expression of spleen HSP70, thymus HSP90 and bursa HSP90 mRNA, as well as SOD activity. *Flavobacteriaceae_uncultured* and ADFI also showed a significant positive correlation ($p<0.05$) (Figure 6B).

DISCUSSION

The production and consumption of animal products could be altered by global warming, which has become one of the primary meteorological factors affecting them. Animals can suffer significant production losses when exposed to high temperatures, since it alters their biological functions. In this study, quails were used as experimental animals to study to the effects of heat stress on production performance, antioxidant functions, *HSP70* and *HSP90* gene expression in immune organs, and caecal microbiota.

Heat shock proteins (HSPs) are a type of protein induced by heat stress, major examples of which are HSP70 and HSP90 (Pirkkala *et al.*, 2001). Physiologically and pathologically, these proteins play a key role in the maintenance of protein homeostasis (Doyle *et al.*, 2019). Currently, they are considered general damaged tissue markers. In quails exposed to heat stress, HSP70

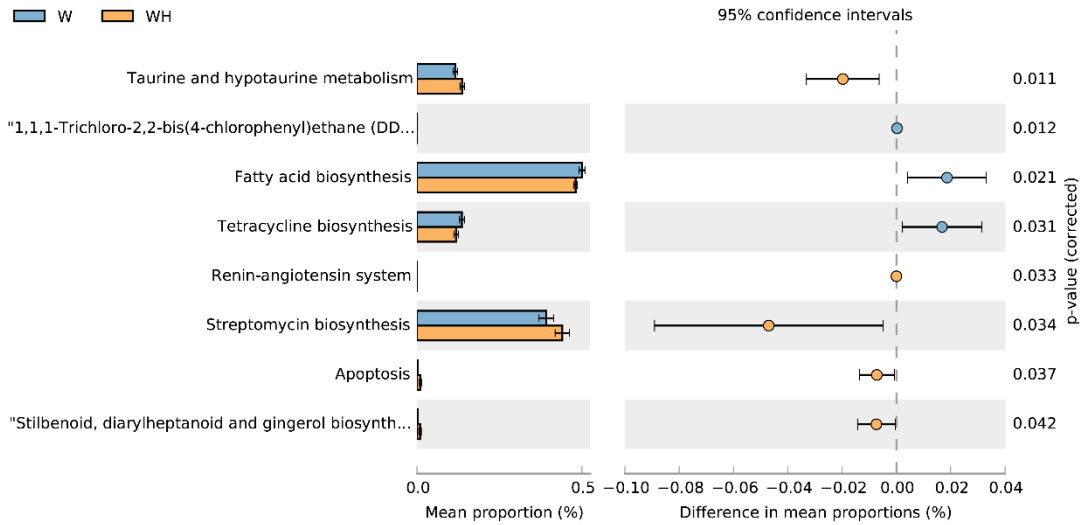

Figure 5 – The Kyoto Encyclopedia of Genes and Genomes (KEGG) L3 orthologs in quails were used to compare the functional properties of caecal metagenomic sequences. A two-sided Welch's t-test was used to determine the differences between the predicted functions. W: control group; WH: heat stress group.

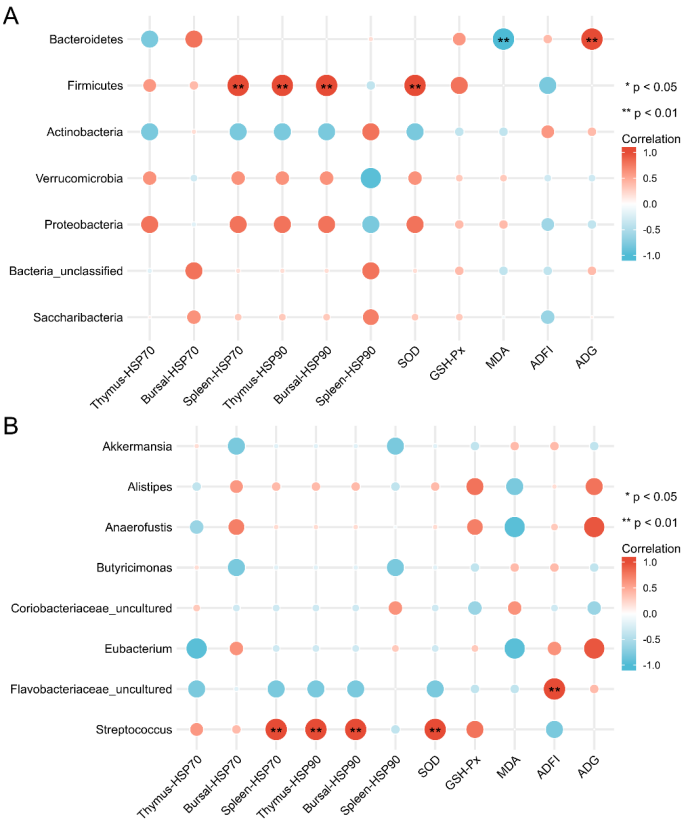

Figure 6 – Correlation analysis between health parameters and caecal microbial composition and the (A) phylum and (B) genus levels. With SPSS Statistics 26.0, Spearman's correlation analysis was conducted, and the results were visualized with R. The red and blue circles represent positive correlation and negative correlation, respectively. HSP, heat shock protein; SOD, superoxide dismutase; GSH-Px, Glutathione peroxidase; MDA, malondialdehyde; ADFI, average daily feed intake; ADG, average daily gain. * $p<0.05$, ** $p<0.01$.

and HSP90 mRNA levels were significantly increased in the thymus, bursa of Fabricius and spleen. This indicates that we successfully modeled heat stress.

Heat stress had overall negative effects on the ADG and F/G of the animals and was consistent with many other studies (Onderci *et al*., 2005; Mehaisen *et al*., 2017). Interestingly, although heat stress reduced feed intake, the groups did not differ significantly, which may be due to increased feeding during the non-stress periods. The significant decrease of ADG during heat stress may be due to reduced food intake or increased blood corticosterone levels that alter energy consumption and promote fat deposition and protein catabolism (Siegel, 1980). Moreover, the heat stress temperature of 32°C inhibits trypsin and amylase activity (Hai *et al*., 2000). This would result in a decrease in nutrient digestibility, and short-term suitable temperatures would not be enough for an effective recovery.

The antioxidant enzyme system is the first barrier of antioxidant defense in animals. It reflects the metabolic levels of reactive oxygen species and the level of tissue damage. This enzyme system includes SOD, CAT and GSH-Px (Blokhina *et al*., 2003). High environmental temperatures cause oxidative stress (OS), which results in an imbalance of the oxidation / antioxidant system. This has already been determined for domestic fowls (Sahin *et al*., 2002). Increasing free radical production can disrupt the redox dynamic balance, causing oxidative damage to lipids, proteins, and DNA, and ultimately leading to cell death (Arnaud *et al*., 2002) and lipid peroxidation in animal plasma and tissues (Naziroglu *et al*., 2000). We found that thermal stress decreased the activity of serum GSH-Px and SOD, and increased MDA levels in quails; which was consistent with another quail study (Kalvandi *et al*., 2019). Moreover, thermal stress can increase plasma MDA contents in quails, which can up-regulate HSP70 levels in liver to protect cells from peroxidation (Kregel, 2002; Kregel & Zhang, 2007).

Nutrient absorption, production performance, and poultry health are highly dependent on the intestinal microbiota (Crisol-Martínez *et al*., 2017; Thomas *et al*., 2019). However, heat stress can destroy the intestinal tract's physical barrier, disrupting the microbiota balance, affecting intestinal digestion and absorption, and leading to decreased productivity (Tian *et al*., 2020; Jin *et al*., 2022). We found that thermal stress decreased the α diversity of caecal microbiota in quails, even though previous studies had reported no changes for α diversity in chicken ileums (Jin *et al*., 2022) and cecums (Xing *et al*., 2019), and in the small bowels of ducks (Tian *et al*., 2020). Interestingly, species richness in the ileal microbiota of broilers increased after thermal stress (Wang *et al*., 2018), while in laying hens a 3-week exposure significantly increased caecal α diversity (Hsieh *et al*., 2017). Thermal stress for 7 days also significantly increased the α diversity of porcine colon microbiota (Hu *et al*., 2021). Therefore, the impact of thermal stress on gut microbiota is complex and varies, and there is a lack of consistency among the experimental results. The OTU abundance for our heat stress group was lower than the controls, indicating a reduced diversity of caecal microbiota consistent with our α diversity results. Heat stress also led to significant changes in the β diversity consistent with earlier researches (Shi *et al*., 2019; Xing *et al*., 2019).

In poultry, there are four main phyla of bacteria in the intestinal microbiota: *Actinobacteria*, *Firmicutes*, *Proteobacteria,* and *Bacteroidetes* (Wei *et al*., 2013; Xiao *et al*., 2017; Wang *et al*., 2018; Tian *et al*., 2020), which is consistent with our control group

populations. After exposure to thermal stress for 7 days, the genus *Alistipes* increased significantly. *Alistipes* can produce short-chain fatty acids (SCFA) such as propionic, isobutyric, isovaleric, and acetic acids (Parker *et al*., 2020). It has been shown that acetic acid and propionic acid can inhibit macrophages from releasing pro-inflammatory cytokines and are potential anti-inflammatory mediators (Zafar & Saier, 2021). The presence of *Alistipes* has a protective effect against colitis in mice (Parker *et al*., 2020), and is a growth promoter for broiler chickens (Torok *et al*., 2011). This Gram-negative anaerobic bacterium also expresses glutamate decarboxylase, which in chickens converts glutamate to gamma-aminobutyric acid (GABA) (Polansky *et al*., 2015). Therefore, there may also be a relationship between the increased *Alistipes* abundance and GABA levels, since GABA addition to the feed of heat-stressed chickens enhances antioxidant and immune functions (Zhang *et al*., 2012; Chand *et al*., 2016) and reduces the influences of heat stress on the digestive enzyme activity, intestinal mucosal absorption, and immune function in chickens (Chen *et al*., 2014). However, it is still unclear whether GABA is secreted into the intestine. In contrast, other studies have shown *Alistipes* to be pathogenic in conditions such as anxiety, myalgic encephalomyelitis/chronic fatigue syndrome, depression, not otherwise specified pervasive developmental disorders, and colorectal cancer (Gagnière *et al*., 2016; Parker *et al*., 2020). Therefore, the exact role of *Alistipes* in heat-stressed quail needs to be deciphered by further experiments.

Through Lefse feature selection, we identified several bacterial genera significantly associated with high temperatures. In particular, *Akkermansia* is a colonizer of the outer mucous membrane of the gastrointestinal in the heat stress group and can utilize mucin as a carbon and nitrogen source. Mucin consumption and goblet cell-regenerated mucin could reach a dynamic balance, and the mucus layer would thus be maintained. (Geerlings *et al*., 2018; Zhang *et al*., 2021). *Akkermansia* also secretes extracellular vesicles (EV) to maintain intestinal homeostasis by binding to Toll-like receptors (TLR) in colonic epithelial cells and regulating the expression of tight junction proteins. This process relieves stress due to inflammatory bowel disease caused by high-fat diets (Caesar *et al*., 2015; Dao *et al*., 2016). In immune cells, phosphatidylethanolamine from *Akkermansia* modulates immune function by regulating TLR2 and TLR2-TLR1 signaling complexes (Bae *et al*., 2022).

We also identified *Anaerofustis* (*Firmicutes*) as a significant genus capable of fermenting carbohydrates into acetic and butyric acids. A decrease in its presence in the gut has been linked to lower levels of these acids, which causes a predisposition to intestinal inflammation (Smith *et al*., 2013; Ma *et al*., 2020). The genus *Butyricimonas* converts glucose into propionic, butyric, isobutyric, acetic and succinic acids. As well as providing energy to intestinal cells, these SCFAs also maintain intestinal barriers and resistance to OS (Sakamoto *et al*., 2014). *Eubacterium* and *Streptococcus* can both produce butyrate, which helps regulate energy balance, immune function, and inhibit intestinal inflammation; as well as convert bile acid and cholesterol, thus promoting their dynamic balance (Ragsdale & Pierce, 2008; Mukherjee *et al*., 2020). It is possible that thermal stress decreases the production of butyrate in the gastrointestinal tract, increases intestinal pH, and causes alterations in the niches of symbiotic and pathogenic bacteria, thus disturbing the homeostasis between dominant and pathogenic bacteria (Deplancke & Gaskins, 2001). According to the Lefse analysis of this study, the caecal microbiota composition in quails also changes as a result of heat stress.

Taurine and hypotaurine are endogenous metabolites that also serve as biomarkers of gastrointestinal injury related to inflammation (Zhou *et al*., 2019). A series of chain reactions causes hypertension when the renin-angiotensin system is activated (Yang & Xu, 2017), significant destroys the gut microbiota balance, damages the intestinal epithelium integrity, alters intestinal permeability, and further activates inflammation (Jin *et al*., 2021). GABA and taurine can antagonize the renin-angiotensin system, indicating that the intestinal microbiota plays an important role in maintaining intestinal barrier integrity (Knepel *et al*., 1980; Kulthinee *et al*., 2019; Li *et al*., 2020; Jaworska *et al*., 2021). Intestinal epithelial apoptosis may be caused by thermal stress, and can also increase mucosal permeability and decrease its barrier integrity (van Grieken *et al*., 2003). We also found reductions in fatty acid and tetracycline biosynthetic pathways and enzymatic functions for DDT degradation that can interfere with intestinal integrity.

In our study, Spearman correlation analysis indicated that *Bacteroidetes* had a significant positive correlation with the average daily weight gain (ADG) and a significant negative correlation with MDA levels. *Bacteroidetes* is a dominant genus in the intestinal tract and promotes normal intestinal metabolism,

nutrient absorption, and ADG, and assists in complex polysaccharide decomposition (Hooper, 2004). Heat stress may result in a decrease in Bacteroidetes abundance, which may explain the ADG decrease. Most *Firmicutes* are SCFA-producing bacteria (Valenzano *et al*., 2015) that utilize HSP70, HSP90, and SOD to alleviate OS induced by thermal stress. Therefore, regulation of the intestinal microbiota may overcome the heat stress. Our future studies will include further analysis of the exact compositions of the intestinal microbiota to the species level, in order to enable more exact functional correlations.

CONCLUSION

This study showed that heat stress reduces the growth performance and antioxidant functions of quail and increases the *HSP70* and *HSP90* levels in the spleen and thymus. The heat stress cycle of 24 - 36ºC decreased the diversity of the caecal microbiota and significantly increased the abundance of *Alistipes* that produce the SCFA necessary for intestinal barrier maintenance. Thermal stress induced alterations of the caecal microbiota were significantly correlated with the expression of the HSP gene, as well as with the levels of ADG and MDA.

AUTHOR CONTRIBUTIONS

Qingming Qin designed the experiment, performed the surgical procedure, analyzed the data, and wrote the original manuscript. Bingjie Ma and Zhili Li performed the experiments. Haigang Wu completed the statistical analysis of experimental data. Jinjiang Zhu and Xueqi Li collected data. Ke Wang reviewed, and Xianguo Yi reviewed and provided funding. All authors edited early drafts, and read and approved the final manuscript.

ACKNOWLEDGEMENTS

This study was supported by the Xinyang Agriculture and Forestry University Innovative Research Team of Application and Healthy Breeding of Poultry Germplasm Resources of Dabie Mountain Area (XNKJTD-013), Henan Province Project of Science and Technology (Grant number:222102110192, 232102110074).

CONFLICT OF INTEREST STATEMENT

This article does not contain any conflicts of interest on the part of the authors.

DATA AVAILABILITY STATEMENT

Please contact the corresponding author if you would like access to the data supporting these findings.

ETHICS STATEMENT

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Xinyang Agriculture and Forestry University and adhered to its standards (XAFU-2021-07055).

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