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Abstract

The study sought to ascertain the impact of resveratrol on the gut microbiome, gut morphology, serum biochemical indicator, and growth performance of geese subjected to heat stress. Three hundred one-day-old male Wanxi white geese were randomly allocated into five groups, each consisting of six replicates of ten geese. The dietary treatments consisted of four heat stress groups receiving 0, 200, 500, or 1,000 mg/kg of resveratrol, alongside a control group maintained at room temperature without resveratrol. The duration of the experiment was 35 days. The results indicated that the final body weight of the heat stress group significantly diminished ($p < 0.05$). The body weight disparity with the control group was nullified following the administration of resveratrol ($p > 0.05$). The control group's average daily feed intake exceeded that of the heat stress group, while the 500 mg/kg and 1,000 mg/kg resveratrol groups under heat stress exhibited no difference in intake ($p > 0.05$). The concentrations of alkaline phosphatase and aspartate aminotransferase were markedly elevated, whereas albumin and glucose levels were substantially diminished in the heat stress group ($p < 0.05$); however, these alterations were effectively alleviated by the administration of 500 mg/kg or 1,000 mg/kg of resveratrol. Concerning antioxidant indicators, malondialdehyde levels were markedly elevated, whereas glutathione peroxidase and total antioxidant capacity were significantly diminished in the heat stress group relative to the control group ($p < 0.05$). Nonetheless, these markers exhibited substantial enhancement when supplemented with 500 mg/kg or 1,000 mg/kg of resveratrol. Heat stress diminished the height of jejunal villi and the ratio of villus height to crypt depth. Supplementation with 500 mg/kg or 1,000 mg/kg of resveratrol significantly diminished the prevalence of Proteobacteria and enhanced the population of butyrate-producing

bacteria, including *Butyricicoccus* and *Prevotella*. In conclusion, dietary supplementation of 500 mg/kg or 1,000 mg/kg of resveratrol during heat stress markedly improved growth performance, enhanced serum antioxidant parameters and intestinal morphology, reduced the prevalence of Proteobacteria, and elevated the activity of Butyrate-producing microorganisms in geese.

Keywords: Antioxidant, Goose, Growth performance, Gut microbiota, Intestinal morphology, Serum index

Introduction

Intensive poultry and livestock farming efficiently satisfies the demand for animal products while markedly improving production efficiency and economic advantages. Nonetheless, it significantly disrupts the natural growth patterns of these animals. Inadequate management of barn conditions, along with shortened growth durations and restricted living space, may result in diminished immunity and adversely affect animal welfare [1]. Consequently, mitigating the environmental effects of animal husbandry is a critical challenge we confront. Heat stress (HS) is a physical environmental stressor that arises when an animal's heat production surpasses its heat dissipation, thereby disrupting normal physiological functions and causing cellular damage [2]. In poultry, HS induces multiple physiological alterations, including oxidative damage, acid-base imbalance, and immune function suppression. This results in diminished feed consumption, decreased feed conversion efficiency, weight reduction, inferior meat quality, and heightened vulnerability to diseases, ultimately elevating mortality rates [3]. Although the impacts of HS on pigs, chickens, and cattle are extensively documented, research on geese is relatively scarce [4]. Geese possess insulating feathers; however, their thin skin is devoid of

sweat and sebaceous glands and has a limited distribution of blood vessels, rendering them especially vulnerable to heat due to inadequate thermoregulatory functions [5]. Traditionally, geese depend on aquatic foraging, which aids in alleviating HS. Nevertheless, the transition to more intensive and high-density farming practices is causing substantial losses among even typically resilient waterfowl due to HS, presenting a significant obstacle to the progress of the poultry industry today [6].

Resveratrol (RES), a natural polyphenolic compound present in grapes and peanuts, exhibits anti-glycation, anti-oxidative, anti-inflammatory, therapeutic, and immune-modulating properties akin to those of other plants [7]. RES is lipophilic and is swiftly absorbed by the body, accumulating in the brain, heart, lungs, testes, liver, kidneys, and intestines [8]. RES is extensively utilized in the medical and cosmetic sectors [9] and is progressively employed in animal production, chiefly for its anti-HS and antioxidant attributes [10]. Studies indicate that administering 300 mg/kg of RES to plants can enhance superoxide dismutase (SOD) activity, SOD2 mRNA expression, and malondialdehyde (MDA) levels in the pectoral muscle [11]. In broiler diets, the inclusion of 300 or 600 mg/kg of RES enhances antioxidant capacity, intestinal morphology, and microbial equilibrium [12]. RES can also activate the Silent Information Regulator 1 signaling pathway to alleviate oxidative and inflammatory damage in the duodenum of ducks caused by HS [13]. Nonetheless, the effects of RES on geese, specifically its antioxidant and immune characteristics under HS, remain undocumented. This experiment aimed to examine the effects of different RES levels on growth performance, organ indices, intestinal morphology, serum biochemical indicators, and gut microbiota in geese subjected to HS.

Materials and Methods

Ethical approval

The protocols established by the Chinese Council on Animal Care were adhered to during the animal treatments. The Animal Care and Use Committee of West Anhui University approved all animal trials (Approval no: SYDW-P20210823021).

Experimental materials

We obtained RES from Shanghai Jiayi Biotech Co., Ltd., which exhibited a purity level surpassing 98%. Three hundred one-day-old Wanxi white geese were acquired from Anhui Wanxi Goose Original Breeding Co., Ltd. The geese were randomly allocated into five dietary treatments, each subjected to distinct dietary treatments, with each group comprising 6 replicates of 10 geese per replicate. Each replicate of 10 geese was housed in the same pen and served as the basic experimental unit for data analysis. Each pen measured 1.2 m × 1.2 m × 1 m. Nipple drinkers, spaced 20 cm apart, were installed in every pen, with their height adjusted according to the geese's growth to ensure easy access to water. Regular water quality checks were conducted to ensure compliance with animal drinking water standards. Geese were regularly vaccinated, and their health was continuously monitored. The experimental groups comprised four groups of geese exposed to heat stress (HS), with each group fed a experimental diet supplemented with RES at concentrations of 0, 200, 500, and 1,000 mg/kg, respectively. The experimental groups comprised four HS groups, each supplemented with RES at concentrations of 0, 200, 500, and 1,000 mg/kg, respectively. Furthermore, a control group (Con) was sustained under standard room temperature conditions without HS or RES

supplementation. All geese were provided experimental diet designed in accordance with the National Research Council 1994 and modified based on the Chinese goose feed formulation (Table 1) [14]. The experiment was performed in the animal facility of Wanxi College, where the geese had unrestricted access to feed and water. A one-week acclimatization phase was succeeded by a four-week experimental phase. The Con group was maintained in an environment at (21 ± 1) °C and (60 ± 5) % relative humidity, whereas the other four groups experienced cyclic HS induced by warm air heaters for temperature elevation and misting for humidity, monitored using a hygrometer. HS was administered daily for 12 hours, commencing at 08:00 with a temperature increase to approximately 33 °C by 09:00, sustained at (33 ± 2) °C until 21:00, subsequently decreased to about 22 °C by 22:00, and maintained at (22 ± 1) °C until the subsequent day at 08:00. Illumination was regulated through a synthesis of artificial and natural light sources.

Growth performance

Geese deprived of food for 12 hours prior to being weighed at both the commencement and conclusion of the trial to ascertain the average daily gain (ADG). Daily feed intake was assessed by weighing the diet supply added before 8:00 and the residuals at 20:00. The feed-to-gain ratio (F/G) and average daily feed intake (ADFI) were assessed.

Indices of organs

Cervical dislocation was employed for the euthanasia of the geese, and the organs, including the liver, spleen, gizzard, glandular stomach, intestines, kidneys, Fabricius bursa, and heart, were excised and measured. The organ weight in grams per kilogram of body weight constitutes the immunological organ scale.

Blood biochemical properties

Following the experiment, a male and female goose from each duplicate, closely aligned in weight and after a 12-hour fast, underwent blood sampling from the wing vein (5 mL). For biochemical assays of total protein (TP), albumin (ALB), globulins (GLOB), alkaline phosphatase (AKP), aspartate aminotransferase (AST), glucose (GLU), blood urea nitrogen (BUN), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) The serum was centrifuged at 3,000 revolutions per minute for at least 10 minutes, and the resultant product was stored at -20°C.

Intestinal morphology

Segments of the jejunum and ileum have been procured and preserved in 4% paraformaldehyde, with the fixative replaced every 24 hours until clarity is achieved. Subsequent to ethanol dehydration, the tissues were embedded, sectioned, and stained with hematoxylin and eosin. The ratio of villus height (VH) to crypt depth (CD) (VH/CD) was calculated after measuring VH and CD under a microscope.

Gut microbiota

Cecal content was obtained for microbiota analysis by Shanghai Ouyi Biomedical Technology Co., LTD. DNA was extracted utilizing the DNA extraction kit (Tiangen, China) to guarantee the purity and concentration of the DNA.The V3-V4 region of the 16S rRNA gene was amplified utilizing the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR reaction mixture (15 μL) comprised

Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 2 μM of each primer, and 10 ng of template DNA. PCR products were combined in equimolar concentrations according to the concentration of each product. The aggregated products were subsequently purified, and the desired bands were extracted utilizing a gel extraction kit. The sequencing libraries were generated via the PCR-free method, and their quality was evaluated using the Qubit® 2.0 Fluorometer and real-time PCR. Sequencing was conducted on an Illumina NovaSeq 6000 platform (paired-end 250 bp) for 16S rRNA gene analysis.

Data analysis

Raw sequencing data underwent processing with QIIME2 for quality control, encompassing the merging of paired-end reads, trimming, denoising, and chimera elimination. FLASH (version 1.2.7) was utilized to merge paired-end reads, while Fastp (version 0.23.1) was employed to filter the raw reads. Chimeric sequences were eliminated to acquire purified data. The UPARSE algorithm (v7.0.1001) was utilized to cluster sequences into Amplicon Sequence Variants (ASVs). Species annotation and classification of ASV sequences were conducted. The alpha diversity indices were computed using QIIME (v2.0), and the diversity analysis was visualized with R software (v2.15.3). Alpha diversity analysis evaluates microbial diversity in environmental samples, reflecting community richness and variability. The abundance is assessed using indices such as Chao1 and Observed species; diversity is quantified by Shannon and Simpson indices; phylogenetic variation is indicated by Faith's PD index. Pielou's evenness index indicates the consistency among samples. Linear discriminant analysis Effect Size (LEfSe) was employed with extensive data on the top 30 genera to examine species composition variations among samples and identify marker species for all groups.

The experimental data collected from different aspects of this study, including growth performance, serum biochemical indicators, and intestinal morphology were organized using Excel and analyzed through one-way ANOVA and LSD post-hoc testing utilizing SPSS 26.0 software. The results were presented as mean ± SEM, with statistical significance defined by $p < 0.05$.

Results

Growth performance

Table 2 indicates no difference in the initial body weights among the five groups ($p < 0.05$). The final body weight of the HS group was lower than that of the Con group ($p < 0.05$); however, there was no difference in body weights between these groups and the Con group following the addition of RES. A trend of diminished ADG was observed in the HS group, while a trend of elevated ADG was noted with increased levels of RES. The ADFI of the HS group was inferior to that of the Con group, and HS + 200 mg/kg RES did not enhance ADFI ($p < 0.05$). No differences were observed in the F/G ratio among the groups ($p > 0.05$), and the ADFI of the HS + 500 mg/kg RES and HS + 1,000 mg/kg RES groups did not differ from the Con group ($p > 0.05$).

Organ indices

Table 3 demonstrates that the heart index, muscular stomach index, and glandular stomach index exhibited no differences among the groups. The liver index in the HS group was reduced compared to the Con group ($p < 0.05$). However, no distinction was observed between the Con group, HS + 500 mg/kg RES, and HS + 1,000 mg/kg RES groups. The indices of the Fabricius and Thymus did not vary between the groups. The RES-supplemented groups did not differ

from the Con group; however, the spleen index was lower in the HS group compared to the Con group ($p < 0.05$).

Serum biochemical parameters

The TP and ALB levels were the lowest in the HS group (Table 4), with the ALB level lower than that of the Con group ($p < 0.05$). The TP and ALB levels were elevated in the HS + 500 mg/kg RES and HS + 1,000 mg/kg RES groups relative to the Con group. The AKP and AST levels were elevated in the HS group relative to the Con group ($p < 0.05$), whereas the RES-supplemented groups exhibited no difference from the Con group. In comparison to the Con group and the HS + 500 mg/kg RES group, the GLU levels were diminished in the HS group and the HS + 200 mg/kg RES group. The results indicated no differences among the groups for the remaining variables.

Serum antioxidant indicators

Table 5 presented the serum antioxidant indicators. The MDA levels in the HS + 500 mg/kg RES group were the lowest, while the levels in the other groups were higher ($p < 0.05$). The trends of GSH-Px and T-AOC became similar, with the HS group exhibiting a decline relative to the Con group ($p < 0.05$), while no differences were noted between the other groups and the Con group.

Intestinal morphology

Table 6 indicates no difference in the jejunum between the Con group and the HS + 1,000 mg/kg RES group. No differences were observed in the VH between the HS + 1,000 mg/kg RES group and the Con group ($p < 0.05$). The VH was diminished in the HS group compared to the HS + 200 mg/kg RES and HS + 500 mg/kg RES groups. The CD of the Con group was

lower than that of the HS groups ($p < 0.05$), and the CD of the HS + 500 mg/kg RES was the lowest. No differences were noted between the HS + 200 mg/kg RES and HS + 1,000 mg/kg RES groups. The VH/CD ratio in the HS + 500 mg/kg RES group was the highest. In the ileum, the VH of the Con group was greater than that of the HS and HS + 200 mg/kg RES groups ($p < 0.05$), while no differences were noted among the other groups. No differences were observed in the CD and VH/CD ratios between the groups.

Cecal microbiota alpha diversity

The Chao1 and Observed species indices for the Con group, HS + 500 mg/kg RES group, and HS + 1,000 mg/kg RES group exceeded those of the HS group (Figure 1) ($p < 0.05$).The HS + 1,000 mg/kg RES group exhibited the highest Shannon, Simpson, and Pielou's evenness indices, which differed from the HS group ($p < 0.05$). The phylogenetic diversity exhibited no differences between the Con and HS groups, while Faith's PD index was higher in the Con group ($p < 0.05$) compared to the HS group.

Cecal microbiota beta diversity

Beta diversity is evaluated through principal coordinate analysis (PCoA) utilizing the Bray-Curtis distance to assess structural variations in microbial communities among groups (Figure 2A). The PCoA indicated that the Con and HS + 1,000 mg/kg RES groups exhibited distinct clustering with differences, while the HS + 500 mg/kg RES group demonstrated increased dispersion within the group. The HS and HS+200 mg/kg RES groups exhibited overlapping samples, signifying minimal differences. Furthermore, an NMDS assessment indicated a stress score of less than 0.2 (stress = 0.17), signifying variability among samples, which serves as a crucial indicator of sample heterogeneity (Figure 2B). The sample overlaps between the HS

and HS + 200 mg/kg RES categories corresponded with the PCoA values, signifying reduced variance among them.

Microbial composition analysis

A stacked bar chart was created to examine variations in microbial abundance across different phyla within each group, based on the relative abundance of bacteria. An analysis of the ten most prominent groups within the phylum and genus level revealed that Firmicutes exhibited the highest abundance (27.84% - 54.46%), succeeded by Proteobacteria (7.05% - 43.08%), Bacteroidetes (3.66% - 35.09%), and Cyanobacteria (0.21% - 16.76%) (Figure 3A). In the HS group, the prevalence of Proteobacteria was maximal, diminishing with escalating RES supplementation, whereas Bacteroidetes exhibited an inverse pattern, being reduced in the Con group and increasing with RES supplementation. Subsequent to *Cupriavidus*, *Acinetobacter*, *Lactobacillus*, *Pseudomonadaceae*, *Pseudomonas*, *Subdoligranulum*, *Oscillospira*, *Faecalibacterium*, *Flavobacterium*, and *Prevotella* demonstrated a notable prevalence at the genus level (Figure 3B). *Lactobacillu*s was elevated in the Con group relative to the other samples.

Differences in genus composition

The Con group exhibited multiple indicators, including *Lactobacillus*, *Acinetobacter*, *Ochrobactrum*, *Streptomyces*, *Rothia*, *Methylobacterium*, *Acetobacter*, *Caulobacter*, and *Rhizobium*. The biomarkers of the HS group comprised *Cupriavidus*, *Enterococcus*, *Streptococcus*, *Aurantimonas*, *Paracoccus*, and *Agrobacterium*. The HS+200 mg/kg RES group exhibited biomarkers including *Flavobacterium*, *Facklamia*, *Corynebacterium*, *Lactococcus*, and *Arthrobacter*, whereas the HS+500 mg/kg RES group displayed biomarkers

such as *Subdoligranulum, [Ruminococcus], Dorea, Butyricicoccus,* and *Slackia*. The biomarkers of the HS+1,000 mg/kg RES group comprised *Prevotella, Barnesiella, Akkermansia,* and *Butyricimonas*.

Discussion

HS directly reduces poultry feed consumption, resulting in reduced meat production and impaired growth performance [15,16]. Elevated temperatures activate the hypothalamus, the thermal regulation center in avians, influencing the expression of appetite-related hormones, including neuropeptide Y. This reduction directly affects feed consumption [17,18]. Research indicates that for poultry, a 1°C rise in environmental temperature from 21 to 30°C leads to an approximate 1.5% reduction in feed intake, whereas a temperature increase from 32 to 38°C results in a decrease of about 4.6% in feed intake [19]. Furthermore, HS diminishes gastrointestinal motility and compromises intestinal integrity, thereby impairing food digestion and absorption, which indirectly influences feeding behavior [20]. Elevated panting and water consumption behaviors under HS conditions further diminish the frequency of feeding behavior [21]. Enhancements in intestinal function may also elevate feed consumption. Studies demonstrate that the inclusion of 0.2% RES in the diet can maintain gut health, reduce pathogenic microbial levels in piglets' intestines, and enhance feed efficiency [22]. Likewise, RES can enhance intestinal morphology and mitigate jejunal mucosal injury by modulating the expression of heat shock proteins, epithelial growth factors, and transcription factors [23]. Our experiment revealed that HS significantly decreased the final weight of geese by 7.26% and ADFI by 16.44%. The addition of 500 mg/kg or 1,000 mg/kg of RES enhanced ADFI, ADG, and F/G ratios. Decreased feed intake, subsequent weight gain, reduced feed efficiency, and

diminished metabolic rate are evident adverse effects of HS on chickens [24]. Chickens subjected to cyclic HS at 42 days of age show a 8.6% decrease in food intake, a 15.4% reduction in weight gain, and an 8.5% increase in F/G [25]. Supplementing the feed of Silkie chickens with RES can mitigate the decline in growth performance under cyclic HS [26].

The organ index is a crucial metric indicating the developmental status of organs. This study discovered that HS diminished the liver and spleen indices in geese. The liver, as the largest metabolic and detoxifying organ, is particularly vulnerable to stressors that can disturb metabolic homeostasis in both the liver and the entire organism [27]. The serum liver function results indicated that HS elevated AST levels. Research indicated that HS treatment resulted in decreased liver weight and induced liver inflammation [28]. The spleen is a vital immune organ in poultry, and reduced organ indices suggest that HS negatively impacts the development of these immune organs, potentially correlating with elevated mortality rates in poultry due to HS. This corresponds with the results of Tang et al., who indicated that HS treatment led to diminished immune organ indices in Wenchang chickens [29]. Moreover, our study demonstrated that the HS+500 mg/RES and HS+1000 mg/RES groups exhibited enhancements in liver and spleen indices post-HS treatment, indicating that RES can partially ameliorate the damage inflicted by HS on organ development in geese. This effect may be associated with RES's capacity to vasodilate, enhance blood circulation, and augment the oxygen and nutrient delivery to the liver and spleen, thereby facilitating their functional recovery [30].

Blood biochemical indicators are essential and efficient markers for evaluating animal metabolism, growth, and immune function. The TP indicates the condition of protein metabolism and nutritional status, reflecting the efficacy of protein synthesis in the body [31].

Research suggest that RES may markedly elevate the TP content in the serum of heat-stressed broiler chickens, implying that RES promotes protein synthesis and modulates hepatic lipid metabolism [32]. This experiment suggests that incorporating RES into the diet generally increase serum TP during HS. The concentration of ALB indicates the liver's ability to synthesize protein [33], whereas GLOB is linked to immune function. Elevated levels of ALB and GLOB signify improved protein utilization and improved immune function [34]. The HS significantly alters blood ALB and GLOB levels, markedly diminishing albumin concentration [35], resulting in reduced plasma colloid osmotic pressure and possible edema; serum GLOB concentration significantly rises [36], which could suggest impaired immune function. The current study identified variations in blood ALB levels among groups, yet observed no effect of HS on GLOB levels, indicating that HS did not result in substantial liver damage in geese in this investigation. However, AST, a key marker of hepatic injury, significantly increased in the HS group, suggesting potential liver dysfunction induced by heat stress. Research indicates that HS can elevate serum ALT and AST levels due to increased oxidative stress, inflammation, and metabolic disturbances in the liver [37]. In our study, we also observed a significant increase in AST and AKP activities under HS, which is consistent with previous findings. The elevated AST levels can be attributed to oxidative stress caused by excessive reactive oxygen species (ROS), which damage liver cells and lead to the release of AST into the bloodstream [38]. Additionally, HS-induced inflammation and altered lipid metabolism, including fat accumulation in the liver, contribute to elevated AST levels [39]. Interestingly, supplementation with RES significantly reduced AST levels in the HS treatment group. RES may mitigate these effects by acting as an antioxidant, reducing oxidative damage, and promoting the clearance of

ROS. Furthermore, RES has anti-inflammatory properties that decrease the release of pro-inflammatory cytokines, thereby protecting the liver from further damage [40]. We also observed differences in serum GLU levels between the groups. The GLU levels in the HS group were significantly lower than in the other groups. This could be attributed to enhanced anaerobic metabolism and increased glycogen utilization during HS [41]. Additionally, HS leads to elevated cortisol levels, which regulate blood glucose by inhibiting insulin secretion and promoting gluconeogenesis [42]. RES, by activating the AMPK (5' AMP-activated protein kinase) pathway, improves insulin sensitivity and may effectively alleviate the negative effects of HS on glucose metabolism [43].

Oxidative stress occurs in the body as an immediate result of mitochondrial damage induced by HS [44]. Oxidative stress denotes an imbalance between oxidative processes and antioxidative defenses, leading to an accumulation of free radicals or dysfunction of the antioxidative system. These two principal types of free radicals are reactive nitrogen species (RNS) and ROS [45]. RES, owing to its composition of three phenolic hydroxyl groups, can interact with free radicals, thereby directly neutralizing ROS/RNS and demonstrating antioxidant properties [46]. Research indicates that the effect of RES on superoxide anion radicals is dose-dependent [47], and by enhancing the activity of antioxidant enzymes, RES also augments its capacity to combat free radicals. Significantly reducing the MDA level and enhancing the synthesis of GSH-Px, SOD, and CAT, the inclusion of RES in poultry feed effectively mitigates oxidative damage induced by HS [48]. RES predominantly stimulates the nuclear factor *Nrf2*, thereby augmenting the expression of antioxidant enzymes [49]. Furthermore, it reduces oxidative damage by activating the *SIRT1*/*FoxO1* signaling pathway.

FoxO1, a ubiquitously expressed nuclear transcription factor in diverse tissues and organs, is crucial for the *SIRT1*-mediated overexpression of MnSOD [50]. Our study revealed that HS markedly diminished antioxidant enzyme activity, elevated MDA levels, and reduced GSH-Px and T-AOC levels. RES supplementation significantly enhanced these metrics, with doses of 1,000 mg/kg yielding levels superior to those in the Con group, unequivocally illustrating RES's ability to scavenge ROS and avert tissue damage [51]. Consequently, as a prospective antioxidant, RES, a phenolic compound, may enhance the antioxidative capacity of geese via its free radical scavenging properties.

HS inflicts considerable pathological damage to the duodenum, jejunum, and ileum of animals, characterized by epithelial cell detachment, submucosal edema, and villous atrophy [52]. This is probably attributable to diminished gastrointestinal blood flow during hyperthermia, resulting in prolonged inadequate circulation and simultaneous edema [53]. The elevation of gastric acid and pepsin production, along with decreased mucus secretion, intensifies epithelial cell shedding, inhibits protein synthesis, and reduces the renewal rate and barrier function of the intestinal epithelium [54]. The pathological damage to the intestinal mucosa induced by HS can severely impair the digestive and absorptive functions of the small intestine, markedly diminishing productive performance. VH and CD are essential indicators of alterations in intestinal morphology. Typically, CD indicates the rate of cell proliferation, as cells persistently migrate and differentiate from the base of the crypts to the tips of the villi, forming absorptive intestinal villus cells to replace those that are lost. Multiple studies demonstrate that the VH/CD ratio can thoroughly represent the functional condition of the small intestine, with an elevation in this ratio signifying improved digestive and absorptive

capabilities [55]. Research indicates that HS adversely affects the morphological development of the duodenum, jejunum, and ileum in weaned piglets, reducing VH, decreasing the VH/CD ratio, and markedly increasing CD in the duodenum [56]. This experiment demonstrated that RES significantly enhanced the VH/CD ratio in the jejunum of geese, thereby facilitating intestinal digestive function.

Polyphenols are essential modulators of intestinal microbial composition and diversity, influencing oxidative stress and metabolism, thus providing advantages to the host [57]. We performed 16S rDNA analysis of the cecal microbiota to evaluate the influence of RES on the gut microbiota of geese subjected to HS. Alpha diversity is regarded as an indicator of host health and stability [58]. This study demonstrated that HS significantly reduced the diversity and complexity of the intestinal microbiota, whereas RES supplementation resulted in notable enhancements in a dose-dependent manner. These results align with the findings of Zhuang et al., which demonstrated that dietary RES modified the alpha diversity of the gut microbiota [59]. Nonetheless, alternative studies have demonstrated no significant effects [60,61], potentially due to variations in dosage or species.

Firmicutes, *Bacteroidetes*, and *Proteobacteria* are the predominant bacterial phyla in the intestines of both pigs and humans [62]. We discovered that these three bacterial types were also the most prevalent in the goose gut microbiota. Stress in animals can induce the proliferation of pathogens such as *Proteobacteria* and *Campylobacter*, as well as result in inadequate nutrient absorption and inflammatory responses [63,64]. Prior research indicates that the majority of bacteria within the phylum *Proteobacteria* can induce chronic intestinal inflammation and injury [65]. In this study, the richness of *Proteobacteria* significantly

increased in the HS group and markedly decreased in the RES groups, implying that dietary RES may alleviate intestinal damage in geese by inhibiting pathogen proliferation. At the generic level, we noted the greatest abundance of *Lactobacillus* in the Con group, which additionally functioned as a biomarker for this group. Research demonstrates that HS can diminish the prevalence of various bacteria within the phylum *Firmicutes*, including *Clostridium* and *Lactobacillus* [66]. Notably, RES supplementation did not markedly enhance the prevalence of *Lactobacillus*, potentially linked to the pH levels under HS [67]. In the HS+1,000 mg/kg RES group, we noted an increased prevalence of *Prevotella*, recognized for its metabolic production of acetate, butyrate, and lactate, which bolster immune function and enhance intestinal health [68]. Other genera with greater abundance, including *Barnesiella*, *Akkermansia*, and *Butyricimonas*, also generate short-chain fatty acids, resulting in analogous effects. Biomarkers in the HS+1,000 mg/kg RES group comprised *Dorea* and *Butyricicoccus*, proficient producers of short-chain fatty acids, particularly butyrate [69]. This demonstrates that, even under HS, 500 mg or 1,000 mg/kg of RES significantly increased the population of certain butyrate-producing bacteria in the goose cecum. This may pertain to RES's antioxidant characteristics, which mitigate oxidative stress and preserve intestinal cell integrity, thus fostering a more stable environment for gut microbiota [70]. Moreover, butyrate functions as an energy substrate for intestinal epithelial cells, aids in the attenuation of inflammation, and facilitates the regeneration of intestinal cells, thereby enhancing gut stability and fostering a beneficial cycle [71]. Furthermore, RES may serve as a fermentable substrate for particular microbes, such as butyrate-producing bacteria, enabling these bacterial strains to utilize RES or its metabolic byproducts, thereby enhancing their prevalence in the intestine.

Conclusion

In conclusion, our experiment under HS conditions markedly diminished the growth performance, serum antioxidant levels, and intestinal villus height of Wanxi white geese. Supplementation with 500 mg/kg or 1,000 mg/kg of RES improved the final weight and feed intake of geese subjected to HS treatment, enhanced their antioxidant indicators and gut morphology, and increased the population of butyrate-producing microorganisms in the cecum. The findings indicate that dietary supplementation with RES can significantly reduce the adverse effects of HS in geese, endorsing its application as a functional feed additive to improve thermal resilience in geese.

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669 **Table 1 Composition and nutrient level of experiment diets (air-dry basis)**

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Items	Content (%)				
Ingredients	Groups				
	Con	HS	HS + 200 mg/kg RES	HS + 500 mg/kg RES	HS + 1,000 mg/kg RES
Resveratrol (98%)	0	0	0.02	0.05	0.10
Corn	67.92	67.92	67.92	67.92	67.92
Soybean meal (43% crude protein)	24.90	24.90	24.90	24.90	24.90
Soybean oil	2.00	2.00	2.00	2.00	2.00
Lys (98%)	0.09	0.09	0.09	0.09	0.09
Met (98%)	0.09	0.09	0.09	0.09	0.09
Thr (98%)	0.00	0.00	0.00	0.00	0.00
Vitamin-mineral Premix[1]	5.00	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrient level[2]					
CP	16.00	16.00	16.00	16.00	16.00
ME (MJ/kg)[b]	12.40	12.40	12.40	12.40	12.40
CF	2.56	2.56	2.56	2.56	2.56
Ca	0.79	0.79	0.79	0.79	0.79
P	0.51	0.51	0.51	0.51	0.51
Lys	0.90	0.90	0.90	0.90	0.90
Met	0.45	0.45	0.45	0.45	0.45
Thr	0.63	0.63	0.63	0.63	0.63
Cys	0.21	0.21	0.21	0.21	0.21

671 [1] One kilogram of the premix contained the following: NaCl 4g, Fe 100 mg, Cu 8 mg, Mn 120 mg, Zn 100 mg, Se

672 0.4 mg, Co 1.0 mg, I 0.4 mg, VA 8330 IU, VB_1 2.0 mg, VB_2 0.8 mg,VB_6 1.2 mg, VB_{12} 0.03mg, VD_3 1440 IU, VE

673 30 IU，biotin 0.2 mg, folic acid 2.0 mg, calcium pantothenic acid 20 mg, niacin acid 40 mg.

674 [2] CP (Crude protein), ME (Metabolizable energy), CF (Crude fiber)

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Table 2 Effects of RES on growth performance of geese under heat stress

Items[1]	Groups[2]					SEM	*p*-value
	Con	HS	HS + 200 mg/kg RES	HS + 500 mg/kg RES	HS + 1,000 mg/kg RES		
Inital BW, g	1836.46	1847.58	1831.21	1873.31	1849.31	20.39	0.937
Final BW, g	4241.88^{a}	3933.73^{b}	4050.44^{ab}	4171.00^{a}	4206.38^{a}	32.61	0.015
ADG, g/d	68.73	59.60	63.41	65.65	67.34	1.09	0.069
ADFI, g/d	311.25^{a}	260.06^{c}	268.99^{bc}	293.15^{ab}	297.80^{a}	5.13	0.003
F/G	4.54	4.37	4.25	4.51	4.35	0.06	0.570

[1] BW (body weight), ADG (average daily gain), ADFI (average daily feed intake), F/G (feed/gain ratio).

[2] Con = control group; HS = Heat stress group; HS+200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS+500 mg/kg RES = Heat stress supplement 500mg/kg resveratrol group; HS+1,000 mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.

[a, b, c] Different letters superscripts mean significant differences ($p < 0.05$).

Table 3 Effects of RES on organ indexes of geese under heat stress

Items[1]	Groups[2]					SEM	*p*-value
	Con	HS	HS + 200 mg/kg RES	HS + 500 mg/kg RES	HS + 1,000 mg/kg RES		
Heart index, g/kg	6.56	6.70	6.71	7.00	7.34	0.15	0.511
Liver index, g/kg	16.61^{a}	13.59^{c}	14.85^{bc}	15.96^{ab}	16.85^{a}	0.31	0.001
Kidney index, g/kg	5.73	5.27	5.48	6.04	6.23	0.15	0.279
Thymus index, g/kg	2.03	1.83	1.99	2.09	2.04	0.11	0.099
Fabricius index, g/kg	0.79	0.78	0.79	0.84	0.91	0.03	0.548
Spleen index, g/kg	0.64^{a}	0.42^{b}	0.53^{ab}	0.67^{a}	0.68^{a}	0.03	0.048
Muscular stomach index, g/kg	37.96	35.56	40.38	41.21	41.30	0.80	0.096
Glandular stomach index, g/kg	2.94	3.15	2.78	3.18	3.29	0.08	0.291

[1] Organ index = organ weight, g/body weight, kg.

[2] Con = Control group; HS = Heat stress group; HS+200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS+500 mg/kg RES = Heat stress supplement 500 mg/kg resveratrol group; HS+1,000 mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.

[a, b, c] Different letters superscripts mean significant differences ($p < 0.05$).

Table 4 Effects of RES on serum Biochemical indices of geese under heat stress

Items[1]	Groups[2]					SEM	*p*-value
	Con	HS	HS + 200 mg/kg RES	HS + 500 mg/kg RES	HS + 1,000 mg/kg RES		
TP, g/L	41.17	37.15	38.60	39.15	40.91	0.51	0.057
ALB, g/L	14.90^{a}	12.65^{c}	13.85^{b}	15.00^{a}	15.60^{a}	0.24	0.001
GLOB, g/L	26.27	24.50	24.75	24.15	25.31	0.37	0.433
AKP, U/L	522.17^{b}	610.83^{a}	566.83^{ab}	533.83^{b}	537.67^{b}	9.63	0.015
ALT, U/L	11.33	12.67	15.83	15.17	14.17	0.71	0.250
AST, U/L	10.50^{b}	13.83^{a}	11.17^{b}	10.50^{b}	9.83^{b}	0.42	0.016
BUN, mmol/L	0.55	0.67	0.65	0.60	0.62	0.04	0.884
GLU, mmol/L	10.83^{a}	8.05^{c}	9.45^{b}	10.78^{a}	10.18^{ab}	0.24	0.001
HDL-C, mmol/L	2.90	2.96	3.33	3.14	2.99	0.06	0.167
LDL-C, mmol/L	2.52	2.62	2.65	2.55	2.68	0.05	0.794
TC, mmol/L	4.65	4.75	5.02	4.71	4.80	0.08	0.675
TG, mmol/L	0.76	0.59	0.74	0.71	0.50	0.04	0.115

[1] TP (total protein), ALB (albumin), GLOB (globulins), AKP (alkaline phosphatase), AST (aspartate aminotransferase), GLU (glucose), BUN (blood urea nitrogen), TC (total cholesterol), TG (triglycerides), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol)

[2] Con = Control group; HS = Heat stress group; HS+200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS+500 mg/kg RES = Heat stress supplement 500 mg/kg resveratrol group; HS+1,000 mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.

[a, b, c] Different letters superscripts mean significant differences ($p < 0.05$).

Table 5 Effects of RES on serum antioxidant indices of geese under heat stress

Items[1]	Groups[2]					SEM	*p*-value
	Con	HS	HS + 200 mg/kg RES	HS + 500 mg/kg RES	HS + 1,000 mg/kg RES		
MDA, ug/ml	149.36^{b}	201.57^{a}	140.23^{b}	118.57^{b}	134.24^{b}	9.01	0.030
SOD, ug/ml	6.33	9.16	7.16	8.00	8.33	0.34	0.084
CAT, ug/ml	20.83	22.16	23.17	20.17	18.67	0.73	0.351
GSH-Px, ug/ml	18.30^{ab}	13.74^{c}	16.59^{bc}	15.99^{bc}	20.13^{a}	0.60	0.004
T-AOC, ug/ml	22.00^{a}	13.47^{b}	17.90^{ab}	20.11^{a}	20.00^{a}	0.92	0.027

[1] SOD (superoxide dismutase), CAT (Catalase), GSH-Px (glutathione peroxidase), T-AOC (total antioxidant capacity), MDA (malondialdehyde)

[2] Con = Control group; HS = Heat stress group; HS+200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS+500 mg/kg RES = Heat stress supplement 500 mg/kg resveratrol group; HS+1,000 mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.

[a, b, c] Different letters superscripts mean significant differences ($p < 0.05$).

Table 6 Effects of RES on intestinal morphology of geese under heat stress

Items[1]	Groups[2]					SEM	*p*-value
	Con	HS	HS + 200 mg/kg RES	HS + 500 mg/kg RES	HS + 1,000 mg/kg RES		
Jejunum							
VH, um	1007.23^{a}	886.71^{b}	924.03^{ab}	969.62^{ab}	976.22^{a}	13.21	0.033
CD, um	221.73^{a}	228.94^{a}	206.20^{ab}	187.28^{b}	195.01^{b}	3.69	0.001
VH/CD	4.75^{ab}	4.12^{b}	4.66^{ab}	5.33^{a}	5.12^{a}	0.11	0.007
Ileum							
VH, um	934.10^{a}	802.84^{c}	866.77^{b}	914.08^{ab}	913.61^{ab}	9.02	0.001
CD, um	167.94	164.09	168.60	166.50	169.97	3.16	0.983
VH/CD	6.09	5.12	5.50	5.69	5.59	0.13	0.215

[1] VH (villus height), CD (crypt depth).

[2] Con = Control group; HS = Heat stress group; HS + 200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS + 500 mg/kg RES = Heat stress supplement 500mg/kg resveratrol group; HS + 1,000 mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.

[a, b, c] Different letters superscripts mean significant differences ($p < 0.05$).

Figure legend:

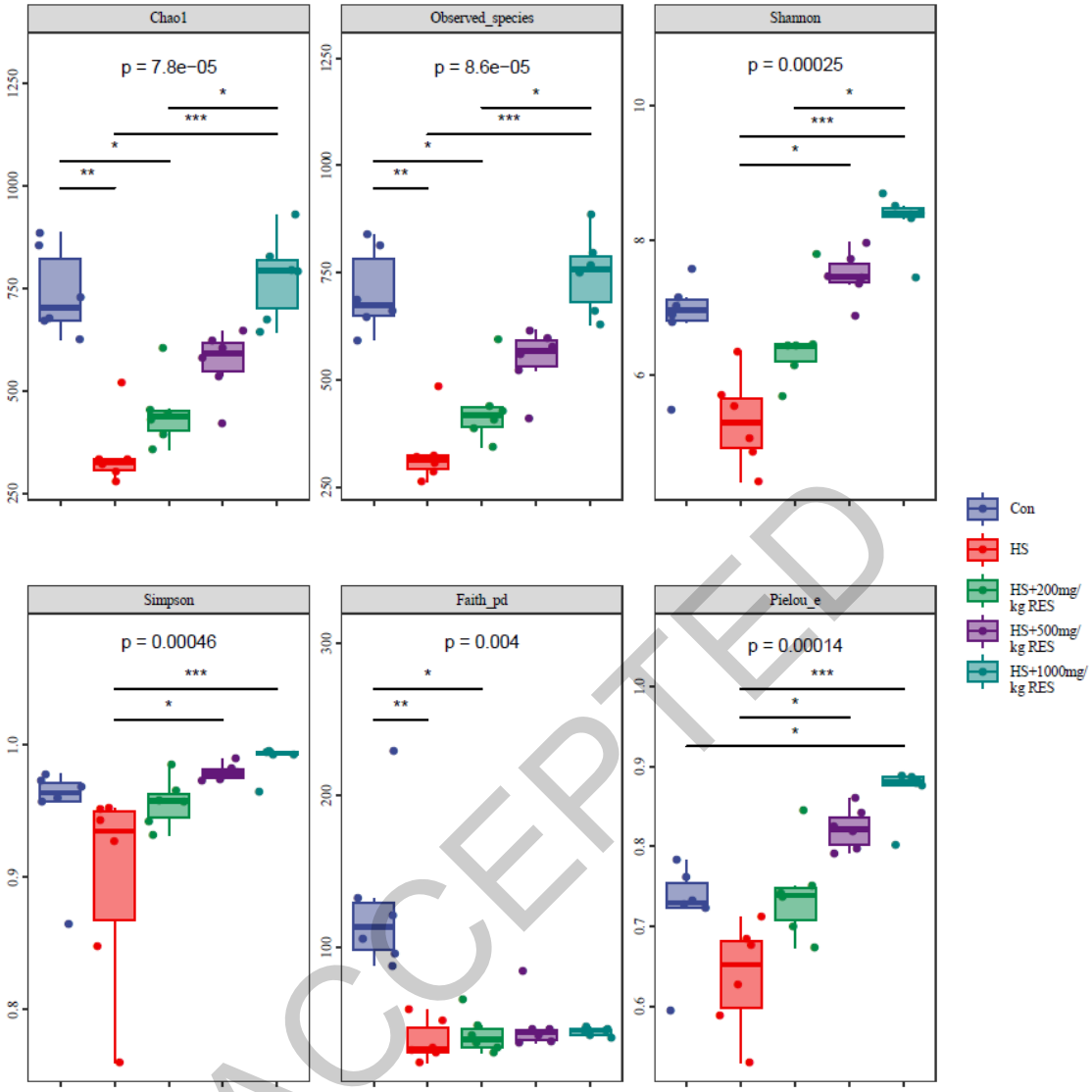

Figure 1. Alpha diversity index. Con = control group; HS = Heat stress group; HS+200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS+500 mg/kg RES = Heat stress supplement 500 mg/kg resveratrol group; HS+1,000mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.

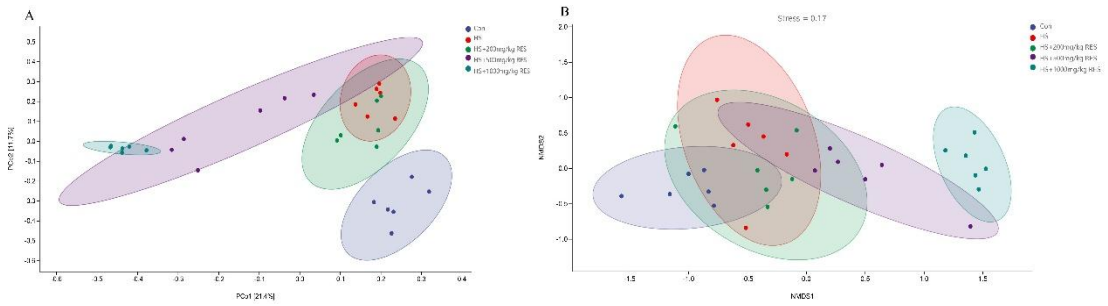

Figure 2. Beta diversity index. (A) Principal coordinate analysis (PCoA) plot of the bacterial community. (B) Nonmetric multidimensional scaling analysis (NMDS) plot of the bacterial community. HS = Heat stress group; HS+200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS+500 mg/kg RES = Heat stress supplement 500 mg/kg resveratrol group; HS+1,000mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.

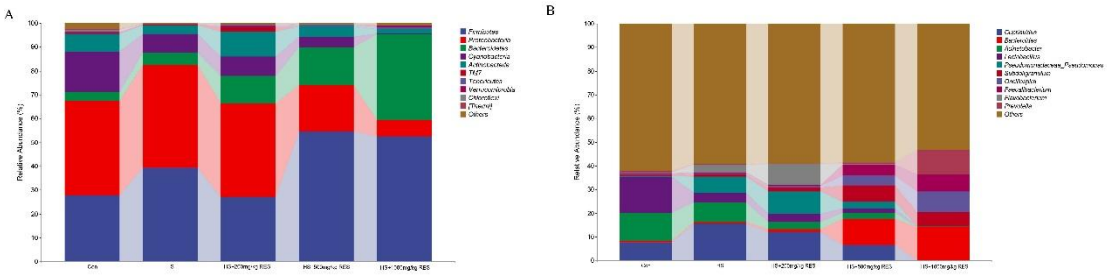

Figure 3. Structure of cecum microbiota. (A) Bar plots at the phylum level. (B) Bar plots at the genus level. HS = Heat stress group; HS+200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS+500 mg/kg RES = Heat stress supplement 500 mg/kg resveratrol group; HS+1,000mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.

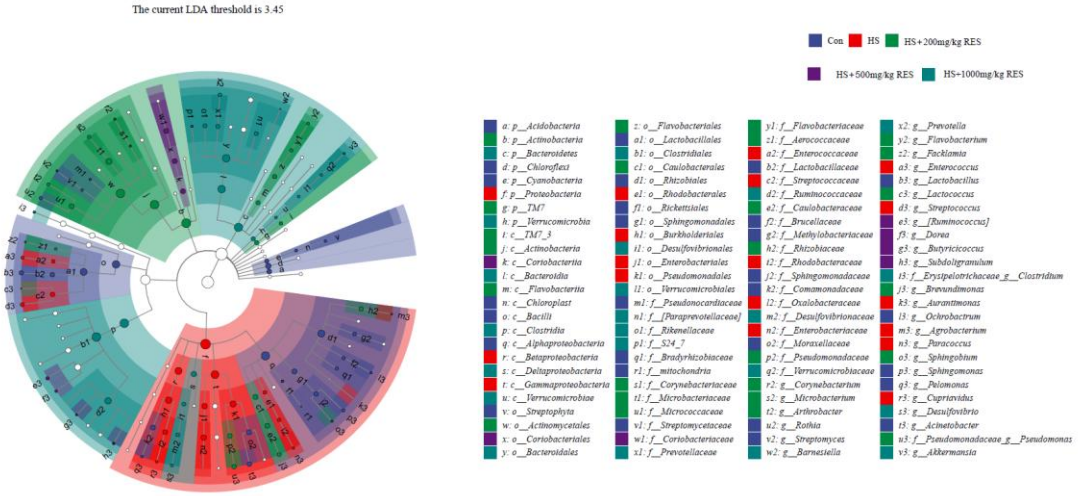

Figure 4. Linear discriminant analysis Effect Size (LEfSe) histogram showing the LDA scores (>3.0) computed for features at the gene level. HS = Heat stress group; HS+200 mg/kg RES = Heat stress supplement 200 mg/kg resveratrol group; HS+500 mg/kg RES = Heat stress supplement 500 mg/kg resveratrol group; HS+1,000mg/kg RES = Heat stress supplement 1,000 mg/kg resveratrol group.