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Original Article

Resveratrol Attenuates Heat-Stress-Impaired Immune and Inflammatory Responses of Broilers by Modulating Toll-Like Receptor-4 Signaling Pathway

■Author(s)

Li Z[I] https://orcid.org/0000-0001-8306-6758
Liu A[I] https://orcid.org/0000-0002-3867-6511
Xu J[I] https://orcid.org/0000-0001-8291-1788
Zhang C[I] https://orcid.org/0000-0002-2346-9624

[I] Department of Animal Science, College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China.

■Mail Address

Corresponding author e-mail address
Cheng Zhang
College of Animal Science and Technology, Anhui Agricultural University, 230036, Hefei, Anhui, China.
Phone: +86 0551-65786326
Email: cheng20050502@126.com

■Keywords

Broiler; heat stress; immune function; resveratrol; signaling pathway.

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ABSTRACT

This study investigated the effect of resveratrol on the immune and inflammatory responses and the mRNA levels of splenic toll-like receptor (TLR)-4 signaling pathway-related genes of broilers under heat stress (HS). One hundred and sixty-two birds were allocated to three groups, each with 6 replicates, for 21 continuous days. The three treatments were as follows: the control group (22 ± 1 °C), the HS (33 ± 1 °C for 10 h d^{-1} and 22 ± 1 °C for the remaining time) group and the HS + resveratrol (400 mg kg^{-1}) group. At the end of the trial, one bird per replicate close to the average body weight (BW) was selected, exsanguinated, and slaughtered. Compared with the control group, the HS treatment decreased (p<0.05) final BW, average daily gain (ADG), average daily feed intake (ADFI), relative weight of bursa of Fabricius and spleen, serum immunoglobulin (Ig) Y, IgA and interleukin (IL)-10 contents, and splenic IL-10 mRNA level, while it increased (p<0.05) feed/gain, mRNA levels of splenic tumor necrosis factor-α (TNF-α), TLR-4, nuclear factor-kappa-B (NF-κB), IL-1β, and IL-6. Compared to the HS group, the HS+resveratrol group exhibited increased (p<0.05) final BW, ADG, relative weight of bursa of Fabricius and spleen, serum IgY, IgA and IL-10 contents, and splenic IL-10 mRNA level, while it exhibited lower (p<0.05) TNF-α, IL-1β and IL-6 contents in serum, and splenic TLR4, TNF-α, IL-1β, and NF-κB mRNA levels. In conclusion, resveratrol prevented a HS-impairment of the immune function of broilers by blocking the abnormal activation of the TLR4 signaling pathway.

INTRODUCTION

Humans and animals have a self-regulating function of body temperature, but heat stress (HS) occurs easily when the thresholds of thermal comfort are below ambient temperature (Hu *et al.*, 2020). With the intensification of global warming, HS is widespread in human life and livestock production, which not only poses a huge threat to the health and welfare of animals, but also affects global livestock production, causing huge economic losses (Lara & Rostagno, 2013; Zhang *et al.*, 2018). Since poultry have no sweat glands and are covered with feathers throughout the body, they are more likely to be affected by HS, which adversely affects endocrine, digestive, and antioxidant functions, therefore hindering the normal growth and development of broilers, and eventually leading to an increase in mortality (Lara & Rostagno, 2013; He *et al.*, 2019).

Previous studies have shown that HS caused harmful effects on the relative weight of immune organs (Zhang *et al.*, 2018), the contents of inflammatory cytokines in serum such as tumor necrosis factor (TNF)-α, interleukin (IL)-2, and IL-4 (He *et al.*, 2018), and the

expression of some inflammatory related genes, including IL-1β, IL-4, TNF-α, and IL-6 (Xu & Tian, 2015; He *et al.*, 2019). However, our understanding of the potential inhibitory effect of HS on the immune function of broilers is limited. As the first member identified in the TLR family, toll-like receptor 4 (TLR4) is the main stress-related biosensor (Mollen *et al.*, 2006). TLR4 can activate nuclear factor kappa B (NF-κB), the main nuclear transcription factor of the natural immunity and inflammatory response (Akira *et al.*, 2006), therefore changing the expression of a series of inflammatory related factors such as TNF-α, IL 6, and IL-1β (Fitzgerald *et al.*, 2003; Mansell *et al.*, 2004; He *et al.*, 2019). Previous studies reported that HS remarkably upregulated the expression of TLR4 (Cheng *et al.*, 2019) and NF-κB (He *et al.*, 2019). Therefore, one of the main reasons for HS-induced innate immunity and inflammatory reaction may be due to the activation of the TLR4 signaling pathway, but this mechanism still needs further research.

There is increasing interest in investigating nutritional manipulation strategies that can prevent the negative effect of HS. Resveratrol, a phytoalexin polyphenolic compound extracted from a variety of plants (e.g., peanuts, berries and grapes), is gaining more and more attention due to its wide range of biological functions, including antioxidant (Zhang *et al.*, 2018), anti-inflammatory (Chen *et al.*, 2018), anti-obesity (Aguirre *et al.*, 2014), and anti-aging (Baur *et al.*, 2006). Previous studies indicated that resveratrol can regulate inflammation through various signaling pathways, including mitogen-activated protein kinases, and phosphoinositide-3 kinases-protein kinase B (Pirola & Froejdoe, 2008). Chen *et al.* (2018) found that resveratrol can alleviate lysophosphatidylcholine-induced inflammation and reduce the release of TNF-α and IL-6 in vascular endothelial cells through inhibition of TLR signaling pathways. Moreover, previous studies have indicated that resveratrol has the ability to regulate TLR4 and NF-κB expression in heat-stressed broilers or ducks (Liu *et al.*, 2016; He *et al.*, 2019; Yang *et al.*, 2021). Given this evidence, we reasonably hypothesized that resveratrol can alleviate HS-induced innate inflammatory and immunity responses of broilers via modulating the expression of the TLR4 signaling pathway. However, relevant information is extremely limited. Therefore, the main objective of the present investigation was to explore the effect of resveratrol on the immune function and the splenic expression of TLR4 signaling pathway-related genes of heat-stressed broilers.

MATERIALS AND METHODS

Experimental Birds and Experimental Design

All procedures used in this experiment were approved by Anhui Agricultural University Animal Ethics Committee (approval code: 2020675). Three hundred 1-day-old male Arbor Acres broilers were routinely fed and immunized. At the age of 21 days, one hundred and sixty two birds with similar body weight were picked and randomly distributed into three treatments: the control group, in which broilers were fed basal diet and reared in a climatic chamber at 22±1 °C 24 h/day, and the HS group and HS + resveratrol group, in which broilers were reared in another climatic chamber at 33±1°C for 10 h per day, from 8:00 AM to 6:00 PM, and 22±1°C for the remaining time, while being fed basal diet and the basal diet with the inclusion of 400 mg kg^{-1}resveratrol, respectively (Zhang *et al.*, 2018). Except for the ambient temperature, other environmental parameters of the different climatic chambers were the same. Each group included six replicates, with 9 birds per replicate. The different experimental treatments lasted for 21 days. Relative humidity was between 60%-70% and the lighting cycle was set at 23 h of light and 1 h of dark per day through the 21-day period. Resveratrol was obtained from Celex Laboratories Inc. (Richmond, British Columbia, Canada). The composition and nutrient levels of basal diets are presented in Table S1. One batch of feed was produced every five to six days, with four batches being produced during the 21-day trial period. In summary, for each batch of feed, 32 g of resveratrol was premixed into 608 g of basal diet to form 5% premix, then the 640 g of 5% premix and 79.36 kg of basal diet were mixed evenly using a v-mixer, with the coefficient of variation being less than 8%. The birds were weighed in replicates at the beginning and the end of the 21-day feeding experimental period. Feed intake was accurately recorded in all replicates. Average daily gain (ADG), average daily feed intake (ADFI), and feed/gain (F/G) were calculated using the equation of Gao *et al.*, (2017).

Sample Collection

At the completion of the 21-day experimental period (the morning of the twenty-second day), one bird per replicate close to average body weight (BW) was chosen and weighed after overnight fasting. Blood samples were collected from the wing veins, and serums were obtained by centrifugation of blood samples at 3500 *g* for 10 min at 4 °C, being kept at -20°C until

Table S1 – Composition and nutrient profile of the basal diets (%, as-fed basis).

Ingredients	1-21 days	22-42 days
Corn	53.10	57.30
Soybean meal	39.20	35.20
Soybean oil	3.76	3.99
Salt	0.30	0.30
Calcium hydrogen phosphate dihydrate	1.90	1.61
Limestone	1.18	1.12
DL-Methionine	0.18	0.10
Choline chloride	0.15	0.15
Premix*	0.23	0.23
Calculated nutrient levels		
Metabolizable energy (Mcal kg^{-1})	2.99	3.05
Crude protein	21.50	20.02
Total Lysine	1.16	1.07
Total methionine	0.50	0.40
Available phosphorus	0.45	0.40
Calcium	1.00	0.90

*The vitamin mix provided (per kg of complete diet): vitamin A, 4000 IU; vitamin D_3, 800 IU; vitamin E, 44 IU; vitamin K_3, 0.5 mg; Thiamine, 1 mg; Riboflavin, 3.75 mg; vitamin B_6, 1 mg; vitamin B_{12}, 15 µg; Niacin, 10 mg; Biotin, 0.2 mg; Pantothenic acid, 12 mg; Folic acid, 1.3mg; Cu, 10 mg as $CuSO_4 \cdot 5H_2O$; Fe, 80 mg as $FeSO_4$; I, 0.6 mg as KI; Zn, 100 mg as $ZnSO_4$; Mn, 25 mg as $MnSO_4$; Se, 0.15 mg as Na_2SeO_3.

subsequence determination. After exsanguination, the selected birds were slaughtered immediately. Spleen, bursa of Fabricius, and thymus were isolated and had their weights recorded to calculate the relative weight of immune organs, as previously described (Zhang *et al.*, 2018). BW and broiler immune organs were weighed with an electronic scale (Maximum: 30 kg; indexing value: 10 g) and an electronic analytical balance (Maximum: 220 g; indexing value: 0.1 mg), respectively. Afterwards, the spleen sample was inserted into liquid nitrogen and then placed at -80°C for the next measurement.

Determination of Serum Immunoglobulin and Inflammatory Cytokine Contents

The serum contents of immunoglobulin (Ig) A, IgM, IgY, TNF-α, IL-1β, IL-2, IL-4, IL-6, and IL-10 were determined by commercially available broiler ELISA diagnostic Kits (Nanjing Jiancheng Biochemistry-Nanjing, China), following the corresponding instructions.

Real-Time PCR

Total splenic RNA was extracted using TRIzol TM Reagent (TaKaRa, Dalian, China), and the concentration and purity of isolated RNA were evaluated using a Nano-Drop 2000 spectrophotometer at 260/280 nm. Extracted samples were then reverse-transcribed into cDNA using a reverse transcription kit (Yeasen, Shanghai, China). The quantification of mRNA was performed on a Real-Time PCR system (Applied Biosystems, Foster City, CA) using the Hieff® qPCR SYBR Green Master Mix (Low Rox Plus) (Yeasen, Shanghai, China). The thermal cycler parameters and the reaction system were according to the settings used in our previous study (Wang *et al.*, 2021). According to the melting curve, each PCR product has only one peak. Primers of β-actin, TLR-4, NF-κB, TNF-α, IL-1β, IL-2, IL-4, IL-6, and IL-10 were designed and synthesized by Generalbiol Corporation (Anhui, China) and are listed in Table S2. Each sample was measured in duplicate and the relative mRNA expression levels of the target genes were determined using β-actin as an internal control through the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Table S2 – Sequences of real-time PCR primers.

Gene	Accession No.	Primer sequence (5′-3′)	Product size (bp)
TLR-4	NM_001030693.1	F: GGCTCAACCTCACGTTGGTA R: AGTCCGTTCTGAAATGCCGT	220
NF-κB	NM_205129.1	F: CACGTTTGGTGCAGTGTCAG R: ATTGACCTTCATGCCCCTCC	253
TNF–α	NM_000594.4	F: CACCACTTCGAAACCTGGGA R: TGTAGGCCCCAGTGAGTTCT	105
IL-1β	NM_204524.1	F: GTACCGAGTACAACCCCTGC R: AGCAACGGGACGGTAATGAA	112
IL-2	NM_204153.1	F: GTCCATTCTGGGACCACTGT R: CCAACGTACATTTTGAGCCCG	107
IL-4	NM_001007079.1	F: GTGCCCACGCTGTGCTTAC R:AGGAAACCTCTCCCTGGATGTC	82
IL-6	NM_204628.1	F: AGGGCCGTTCGCTATTTGAA R: CAGAGGATTGTGCCCGAACT	72
IL-10	NM_001004414.2	F: GGAGCTGAGGGTGAAGTTTGA R: GACACAGACTGGCAGCCAAA	129
β-actin	NM_205518.1	F: TTGGTTTGTCAAGCAAGCGG R: CCCCCACATACTGGCACTTT	127

IL = interleukin; NF-κB = nuclear factor kappa B; TLR = toll-like receptor; TNF-α = tumor necrosis factor-α.

Statistical Analysis

All data were analyzed using SPSS 18.0 software (SPSS, Inc, Chicago, IL) and expressed as mean ± standard error. Each replicate (n=6) served as the experimental unit for growth performance, and individual broiler (n=6) was used as the experimental unit for the other indicators, including organ index, serum immunoglobulin and inflammatory cytokine contents, and relative gene levels. The general linear model was applied ($Yij = \mu + di + \varepsilon ij$; Yij: the observation, μ: the general mean, di: the treatment effect, εij: the random error). One-way ANOVA followed by Tukey test was performed to examine the statistical difference between groups. A nonparametric Kruskal-Wallis test followed by the Student-Newman-Keuls (SNK) test was used to analyze the data when variances were not homogeneous (only IL-2 and IL-4). $p<0.05$ was defined as statistically significant.

RESULTS

Growth Performance

As displayed in Table 1, birds in the HS group had lower final body weight, average daily gain (ADG), and average daily feed intake (ADFI), but with higher feed/gain (F/G) than those in the control group (p<0.05). Birds in the HS + resveratrol group had higher final BW and ADG, and lower F/G than those in the HS group (p<0.05).

Table 1 – Effect of resveratrol on the growth performance of heat-stressed broilers.

Items	Control	HS	HS + resveratrol
Initial BW (g)	849.82±13.95	855.00±16.29	819.33±13.37
Final BW (g)	2340.67±23.63	2055.89±32.69*	2217.83±15.25*#
ADG (g)	70.99±0.71	57.18±1.67*	66.60±1.31*#
ADFI (g)	128.08±0.86	121.89±0.76*	123.82±0.44*
Feed/Gain	1.80±0.01	2.13±0.02*	1.86±0.03#

*=<0.05 versus control and #p<0.05 versus HS in a row. Control: basal diet + normal temperature (22 ± 1 °C), HS: basal diet + heat stress temperature (33 ± 1 °C), HS + resveratrol: basal diet + heat stress temperature (33 ± 1 °C) + resveratrol (400 mg/kg). BW = body weight; ADG = average daily gain; ADFI = average daily feed intake.

Organ Indexes

As shown in Table 2, HS decreased the relative weight of the spleen and bursa of Fabricius compared to the control group (p<0.05). These indexes were increased by resveratrol in broilers subjected to HS (p<0.05).

Table 2 – Effect of resveratrol on organ indexes of heat-stressed broilers.

Items	Control	HS	HS + resveratrol
Spleen (mg g^{-1})	2.88±0.16	1.67±0.08*	2.38±0.18#
Bursa of Fabricius (mg g^{-1})	2.27±0.19	1.56±0.09*	2.05±0.14#
Thymus (mg g^{-1})	3.54±0.15	3.37±0.46	3.69±0.32

*p<0.05 versus control and #p<0.05 versus HS in a row. Control: basal diet + normal temperature (22 ± 1 °C), HS: basal diet + heat stress temperature (33 ± 1 °C), HS + resveratrol: basal diet + heat stress temperature (33 ± 1 °C) + resveratrol (400 mg/kg).

Serum Immunoglobulin Contents

As presented in Table 3, birds under HS had lower serum IgY and IgA contents compared to the control group (p<0.05). These indexes were increased in the serum of heat-stressed broilers by the addition of resveratrol (p<0.05).

Table 3 – Effect of resveratrol on the serum immunoglobulin contents of heat-stressed broilers.

Items	Control	HS	HS + resveratrol
IgY (mg mL^{-1})	4.34±0.45	2.84±0.30*	4.17±0.33#
IgM (mg mL^{-1})	1.83±0.22	1.77±0.12	1.88±0.21
IgA (mgmL^{-1})	1.98±0.11	1.39±0.13*	1.90±0.11#

*p<0.05 versus control and #p<0.05 versus HS in a row. Control: basal diet + normal temperature (22 ± 1 °C), HS: basal diet + heat stress temperature (33 ± 1 °C), HS + resveratrol: basal diet + heat stress temperature (33 ± 1 °C) + resveratrol (400 mg/kg). Ig = immunoglobulin.

Serum Inflammatory Cytokines Contents

The contents of serum inflammatory cytokines in broilers are shown in Table 4. Broilers in the HS group had greater TNF-α, IL-1β, IL-2, and IL-6 contents, and lower IL-10 content than those in the control group (p<0.05). Birds in the HS + resveratrol group had decreased serum IL-1β, IL-6, and TNF-α contents, and an increased IL-10 content compared to the HS group (p<0.05).

Table 4 – Effect of resveratrol on serum inflammatory cytokines contents of heat-stressed broilers.

Items	Control	HS	HS+resveratrol
IL-1β (ng L^{-1})	55.81±2.23	71.51±2.88*	60.46±2.24#
IL-2 (ng L^{-1})	101.38±3.66	112.34±2.39*	102.92±4.58
IL-4 (ng L^{-1})	45.11±1.99	41.07±2.55	44.77±2.45
IL-6 (ng L^{-1})	168.21±5.79	198.45±6.59*	165.53±8.17#
IL-10 (ng L^{-1})	101.00±5.15	75.81±2.59*	91.39±5.13#
TNF-α (ng L^{-1})	80.09±3.47	103.72±5.74*	83.24±4.05#

*p<0.05 versus control and# p<0.05 versus HS group in a row. Control: basal diet + normal temperature (22 ± 1 °C), HS: basal diet + heat stress temperature (33 ± 1 °C), HS + resveratrol: basal diet + heat stress temperature (33 ± 1 °C) + resveratrol (400 mg/kg). IL = interleukin.

Relative Genes mRNA Expression Level in the TLR4 Signaling Pathway

As presented in Table 5, broilers in the HS group had greater TNF-α, IL-1β, IL-6, TLR4, and NF-κB mRNA levels, and a lower IL-10 mRNA level than those in the control group (p<0.05). Birds in the HS + resveratrol group had lower TNF-α, IL-1β, TLR4, and NF-κBm RNA levels, and a greater IL-10 mRNA level than those in the HS group (p<0.05).

Table 5 – Effect of resveratrol on the relative genes mRNA expression level of heat-stressed broilers in the TLR4 signaling pathway.

Items	Control	HS	HS+resveratrol
TLR4	1.00±0.08	2.08±0.20*	1.21±0.18#
NF-κB	1.00±0.10	1.79±0.26*	0.95±0.12#
IL-1β	1.00±0.16	1.61±0.14*	0.97±0.14#
IL-2	1.00±0.13	0.97±0.09	0.82±0.08
IL-4	1.00±0.15	0.74±0.14	0.96±0.11
IL-6	1.00±0.14	1.66±0.26*	1.19±0.15
IL-10	1.00±0.13	0.62±0.10*	0.97±0.08#
TNF-α	1.00±0.14	2.19±0.23*	1.53±0.19*#

*p<0.05 versus control and #p<0.05 versus HS in a row. Control: basal diet + normal temperature (22 ± 1 °C), HS: basal diet + heat stress temperature (33 ± 1 °C), HS + resveratrol: basal diet + heat stress temperature (33 ± 1 °C) + resveratrol (400 mg/kg). IL=interleukin; NF-κB=nuclear factor kappa-B; TLR=toll-like receptor; TNF-α=tumor necrosis factor-α.

DISCUSSION

Due to their greater metabolic heat generation and the lack of sweat glands, poultry are particularly sensitive to HS (Zhang *et al.*, 2017). Therefore, HS

can cause huge economic losses, which is why it is considered to be the most serious problem in broiler production. Previous studies reported that broilers subjected to HS exhibited a significant reduction of BW gain (by 25.37%) and feed intake (by 11.49%), as well as a significant increase of F/G (by 19.33%) (Liu *et al.*, 2014; Safdari-Rostamabad *et al.*, 2017). A lot of researches showed that nutritional manipulation is a practicable method that can alleviate the adverse impacts of HS in broiler production. It was reported that resveratrol could act as a powerful phytoalexin polyphenolic compound to alleviate the HS-induced growth performance descent of broilers, which is manifested in the significant increase of final BW by 3.95% and the remarkable decrease of F/G by 5.17% (Liu *et al.*, 2014; Zhang *et al.*, 2017). Likewise, the present study provided more evidence supporting the protective role of resveratrol on the growth performance of broilers under HS.

In poultry, the spleen is the key peripheral immune organ, constituting the major immune system alongside the bursa of Fabricius and the thymus (John, 1994). They are the vital organs for broiler immune function, taking part in humoral and/or cellular immunity (Liu *et al.*, 2014; Zhang *et al.*, 2018). Many studies have reported that HS led to a significant decrease of organ indexes for the spleen by 15.94% and bursa of Fabricius by 46.23% in broilers (Park *et al.*, 2013; Liu *et al.*, 2014). The results in this work also showed that HS remarkably reduced broilers' relative spleen and bursa of Fabricius weights by 42.01% and 31.28%, respectively. The results above suggest that the development status of immunological organs was adversely affected by HS and that immune function was impaired. Interestingly, previous studies consistently found that resveratrol significantly improved the organ indexes of thymus, spleen, and bursa Fabricius of broilers under HS by 118.33%, 30.17% and 36.84%, respectively, indicating that the unfavourable effect of HS on the immune organ indexes of broilers could be alleviated by the addition of resveratrol (Liu *et al.*, 2014; He *et al.*, 2019). Such positive findings suggest that plant functional components have protective effects against immune organ damage. Again, the present results for the immune organs indicate that resveratrol significantly improved the relative weights of the spleen and bursa of Fabricius of heat-stressed broilers by 42.51% and 31.41%, respectively. These results suggest that the immune status of broilers under HS can possibly be improved by resveratrol.

HS has been reported to restrain the humoral immune response of broilers and reduce the production of antibodies (Bartlett & Smith, 2003; Sohail *et al.*, 2010). IgY, IgA, and IgM are the major classes of immunoglobulins present in domestic chicken, participating in the maintenance of humoral immunity (Ulmer-Franco *et al.*, 2012). Previous studies in broilers found that the contents of IgM (Bartlett and Smith, 2003) and IgY (Bartlett & Smith, 2003; Park *et al.*, 2013) in serum significantly declined under HS, by 43.37% and 27.27%, respectively. Moreover, a recent study reported that the contents of IgY and secretory IgA in the jejunum of broilers were decreased under HS, by 24.99% and 20.40%, respectively (Song *et al.*, 2018). Similarly, our work revealed that the serum contents of IgY and IgA significantly decreased under HS, respectively by 34.56% and 29.80%. A previous study reported that nutritional regulation could reduce the negative effects of HS on jejunal secretory IgA and IgY concentrations (Song *et al.*, 2018). The present study reported a significant increase of IgA and IgY serum contents in broilers under heat stress by using resveratrol (respectively, 36.69% and 46.83%), indicating that the impaired humoral immunity of broilers subjected to HS can be attenuated by it. The dynamic balance of the secretion of immune mediators between anti-inflammatory and pro-inflammatory cytokines plays a major part in the production of immunoglobulins (Macpherson *et al.*, 2008). Therefore, we speculated that the changes in serum immunoglobulins of heat-stressed broilers may result in the imbalance of cytokine secretion, while resveratrol may alleviate the negative effect of HS on the pro-inflammatory and anti-inflammatory cytokine secretion. To prove our assumption, some cytokine contents in serum and its mRNA expression levels in the spleen were further measured. The results we obtained showed that the contents of IL-1β, IL-2, IL-6, and TNF-α in serum, and the mRNA expression levels of IL-1β, IL-6, and TNF-α in the spleen were significantly raised by HS, while the IL-10 content in serum and its mRNA level in the spleen decreased by respectively 24.94% and 38.00%. A previous study also demonstrated that the expressions of IL-1 and TNF-α protein in the spleen were remarkably increased by nearly 280% and 190% under HS (Xu *et al.*, 2017). Moreover, Xu and Tian, (2015) reported that the mRNA levels of IL-2, IL-4, and TNF-α in the bursa of Fabricius, spleen, and thymus rapidly increased under HS. Under high temperatures, the body is prone to oxidative stress. Therefore, oxidative stress induced by HS increases the expression

of pro-inflammatory factors (IFN-γ, TNF-α, and IL-2) and decreases anti-inflammatory factors, including IL-10 expression, ultimately resulting in immune dysfunction (He *et al.*, 2019). There is increasing interest in mitigating the HS-induced inflammatory response via dietary supplementation with certain nutrients, for example Se (Xu & Tian, 2015) and the polysaccharide of Atractylodes macrocephala Koidz (Xu *et al.*, 2017). It is well-known that resveratrol has an anti-inflammatory function (He *et al.*, 2019; Meng *et al.*, 2021). A recent study observed that resveratrol can significantly decrease TNF-α concentration by nearly 12%, and its mRNA level by nearly 63% in the liver of heat-stressed rats (Cheng *et al.*, 2019). The present study shows that resveratrol resulted in significantly lower contents of TNF-α, IL-1β, and IL-6 in the serum (respectively 19.75%, 15.45, and 16.59%) and mRNA level of TNF-α and IL-1β in the spleen (respectively 30.14% and 39.75%), as well as a higher IL-10 content (20.25%) in serum and its mRNA level in the spleen (56.45%), indicating that resveratrol can effectively inhibit the harmful inflammatory response induced by HS.

TLRs are a class of innate immune receptors, of which TLR4 is the major stress-related biosensor (Mollen *et al.*, 2006). NF-κB is the critical nuclear transcription factor of the innate immunity and inflammatory response. TLR4 can activate NF-κB through the myeloid differentiation factor (MyD) 88-independent pathway and the MyD88-dependent pathway, ultimately changing the expression levels of a series of inflammation-related genes including TNF-α, IL-1β, IL-6, and IL-10 (Fitzgerald *et al.*, 2003; Mansell *et al.*, 2004; Mollen *et al.*, 2006). A recent study in rats found that the TLR4 level in the liver of rats was significantly improved under HS, by nearly 40% (Cheng *et al.*, 2019). The research by Liu *et al.* (2016) revealed that the level of NF-κB expression in the jejunum of broilers was significantly increased by HS. Additionally, resveratrol treatment improved immune function by reducing the expression of pro-inflammatory mediators such as IL-1β, IL-6, and TNF-α, thus inhibiting the TLRs/NF-κB pathway (Meng *et al.*, 2021). In this work, results showed that HS up-regulated the mRNA expression level of NF-κB and TLR4 in the spleen by 79% and 108%, respectively. Chen *et al.*(2018) found that resveratrol could modify lysophosphatidylcholine-induced inflammation in vascular endothelial cells by suppressing the TLR4 signalling pathway. Interestingly, this study demonstrated that resveratrol significantly decreased the expression levels of TLR4 by 41.83% and of NF-κB by 46.93%, suggesting that the effect of resveratrol on the suppression of HS-induced inflammatory response of heat-stressed broilers may be mediated via the TLR4 signaling pathway.

CONCLUSION

HS caused immune organ dysplasia and the occurrence of harmful immune and inflammatory responses, which are evidenced by the lower serum immunoglobulins and anti-inflammatory cytokines, and higher serum pro-inflammatory cytokines. Resveratrol could partly prevent the detrimental immune and inflammatory response induced by HS by suppressing the abnormal activation of the TLR4 signaling pathway. The findings of the present study revealed that the use of resveratrol may be a feasible nutritional strategy to prevent HS-impaired immune function. Further study is naturally necessary to clarify the underlying mechanism of action.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this article.

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