INTRODUCTION

High summer temperatures and high stocking densities of chickens have been shown to result in performance reduction, metabolic disorders (Mujahid et al., 2005), immune suppression (Bartlett and Smith, 2003; Mashaly et al., 2004), decreased meat quality (Sandercock et al., 2001; Azad et al., 2010), and increased mortality (Mashaly et al., 2004). Thus, substantial attention has been given to the use of nutritional supplements to combat heat stress. A botanical polyphenolic compound, resveratrol (3,5,4′-trihydroxy-trans-stilbene) is considered an important monomeric bioactive compound that exhibits a strong antioxidant capacity to scavenge free oxygen and lipid radicals (Rubiolo and Vega, 2008). Resveratrol inhibits the formation of glutathione disulfide and maintains glutathione in a reduced state, thereby inhibiting the cellular damage produced by free radical reactions (Hung et al., 2000). It has been previously reported that resveratrol and its derivatives exhibit antioxidant and antimicrobial (bacterial) properties, and that resveratrol prevents cancer cell proliferation (Wolter and Stein, 2002; Yang et al., 2009). In a recent study, resveratrol was shown to protect DNA from oxidative damage (Yan et al., 2012). Therefore, it is plausible to hypothesize that resveratrol may play a critical role in the reduction of cellular malonaldehyde and the reduction of heat stress-induced reactive oxygen species in black-boned chickens.

Animals exposed to heat stress often exhibit a weakened immune system. Studies have shown that natural polyphenolic additives could improve body immunocompetence and scavenge peroxides in immune cells (Bub et al., 2003; Bayer et al., 2004). Heat stress is also

Resveratrol induces antioxidant and heat shock protein mRNA expression in response to heat stress in black-boned chickens

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ABSTRACT This study investigated the effects of dietary resveratrol at 0, 200, 400, or 600 mg/kg of diet on the performance, immune organ growth index, serum parameters, and expression levels of heat shock protein (Hsp) 27, Hsp70, and Hsp90 mRNA in the bursa of Fabricius, thymus, and spleen of 42-d-old female black-boned chickens exposed to heat stress at 37 ± 2°C for 15 d. The results showed that heat stress reduced daily feed intake and BW gain; decreased serum glutathione (GSH), growth hormone, and insulin-like growth factor-1 levels; and inhibited GSH peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) activities compared with birds subjected to thermo-neutral circumstances. Chickens that were fed diets supplemented with resveratrol exhibited a linear increase in feed intake and BW gain (P < 0.001); serum GSH, growth hormone, and insulin-like growth factor-1 levels (P < 0.01); and GSH-Px, SOD, and CAT activities (P < 0.001) compared with chickens that were fed diets without resveratrol during heat stress. In contrast, serum malonaldehyde concentrations were decreased (P < 0.001) in the chickens fed a resveratrol-supplemented diet. Heat stress also reduced (P < 0.05) the growth index of the bursa of Fabricius and spleen; however, it had no effect on the growth index of the thymus. The growth index of the bursa of Fabricius and spleen increased (P < 0.01) upon heat stress and coincided with an increase in supplemental resveratrol levels. The expression of Hsp27, Hsp70, and Hsp90 mRNA in the bursa of Fabricius and spleen were increased (P < 0.01), but those of Hsp27 and Hsp90 mRNA in thymus were decreased (P < 0.01) under heat stress compared with no heat stress. Resveratrol attenuated the heat stress-induced overexpression of Hsp27, Hsp70, and Hsp90 mRNA in the bursa of Fabricius and spleen and increased the low expression of Hsp27 and Hsp90 mRNA in thymus upon heat stress. The results suggest that supplemental resveratrol improves growth performance and reduces oxidative stress in heat-stressed black-boned chickens by increasing serum growth hormone concentrations and modulating the expression of heat shock genes in organs of the immune system.

Key words: antioxidant response, black-boned chicken, heat stress, heat shock protein, resveratrol

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associated with an increase in the gene expression of a series of heat shock proteins in chickens (Mahmoud et al., 2004b; Yu et al., 2008). Heat shock proteins (Hsp) are classified by molecular weight (Noble et al., 2008) and serve as universal cytoprotective proteins that may enhance stress tolerance and increase the survival rate of heat-stressed animals (Latchman, 2001). Recently, it has been suggested that Hsp mRNA expression in animals exposed to acute heat stress is increased compared with animals maintained under normal environmental conditions (Yu and Bao, 2008; Gu et al., 2012). It has also been reported that some polyphenolic supplements can regulate Hsp mRNA expression (Aalinkeel et al., 2008; Sahin et al., 2012); however, the mechanisms underlying heat stress induced in Hsp mRNA expression in the immune organs of black-boned chickens are still unclear. Here, we report the results of a study designed to investigate oxidative damage, growth hormones levels, and insulin-like growth factor-1 responses to resveratrol dietary supplementation in black-boned chickens. Another aim of this study was to investigate resveratrol as a chaperone inducer that may contribute to the regulation of Hsp27, Hsp70, and Hsp90 mRNA expression in the immune organs of black-boned chickens upon exposure to heat stress.

MATERIALS AND METHODS

Birds, Diets, and Experimental Design

Three hundred female Xuefeng black-boned chickens, 28 d of age, free of infectious disease, were obtained from Hunan Songyun Commercial Fowl Company and transferred to the laboratory of the Faculty of Animal Science, Hunan Agricultural University. Resveratrol (≥98% purity) was extracted from Polygonum cuspidatum at the Hunan Engineering and Technology Center for Natural Products. The experiments were conducted between August and September 2012. The chickens were supplied a basal diet and water ad libitum. After a 2-wk adaptation period, the black-boned chickens were individually weighed (the average weight was 293 g) and divided into 5 groups at random; each group of black-boned chickens were further subdivided into 6 replicate groups (10 chickens/replicate) and housed in 6 cages with dimension of 100 × 100 cm. In group one, 60 black-boned chickens were fed a basal diet and maintained at 24 ± 2°C for 24 h/d (normal temperature, NT). The remaining 4 groups which included 240 black-boned chickens were housed in temperature-controlled rooms at 37 ± 2°C for 8 h/d (high temperature, HT; 0930–1730 h) followed by housing at 24 ± 2°C for the remaining 16 h/d. This treatment lasted for 15 d (from d 42 to 57). During the treatment period, the birds of NT group received a basal diet and the birds in the HT groups received 1 of 4 diets: the basal diet or basal diet supplemented with 200, 400, or 600 mg of resveratrol per kg of diet. The basal diet was formulated to meet nutrient requirements (Table 1). On d 57, blood samples and immune organs were taken from one bird per cage for laboratory analysis (30 birds, 6 birds from each of the 5 groups). The protocol for this study was approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University.

Record of Performance

Body weights of black-boned chickens were measured at the beginning (d 42) and the end of the heat stress trial (d 57). Feed intake was recorded daily. Average daily feed intake (FI), average daily BW gain (BWG), and feed/gain (F/G) ratios were calculated.

Immune Organ Growth Index Determination

Black-boned chickens were weighed and killed. The bursa of Fabricius, thymus, and spleen were excised and weighed. Immune organ index (mg/g) was calculated as the immune organ fresh weight (mg)/chicken weight (g) before slaughter.

Measurement of Antioxidant Biomarkers

The serum levels of malonaldehyde (MDA) and glutathione (GSH), and the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in serum were determined according to the instructions provided with the commercial assay kits (Nanjing Jiancheng Biochemical Reagent Co., Nanjing, China). Measurements were obtained via an automated fluorescence instrument (Thermo Fisher Scientific, Waltham, MA). The TBA method was used.

Table 1. Composition and nutrient levels of the basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount, air-dry basis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td>Amount, air-dry basis, %</td>
</tr>
<tr>
<td>Corn</td>
<td>59.92</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>29.37</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>3.24</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.34</td>
</tr>
<tr>
<td>Corn oil</td>
<td>3.28</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.63</td>
</tr>
<tr>
<td>Salt</td>
<td>0.38</td>
</tr>
<tr>
<td>Chloride choline</td>
<td>0.10</td>
</tr>
<tr>
<td>D, L-Methionine</td>
<td>0.08</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin and mineral premix1</td>
<td>0.56</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
<tr>
<td>Nutrient level2</td>
<td></td>
</tr>
<tr>
<td>ME, MJ/kg</td>
<td>12.41</td>
</tr>
<tr>
<td>CP, g/kg</td>
<td>18.65</td>
</tr>
<tr>
<td>Calcium, g/kg</td>
<td>1.03</td>
</tr>
<tr>
<td>Phosphorus, g/kg</td>
<td>0.45</td>
</tr>
<tr>
<td>Lysine, g/kg</td>
<td>0.85</td>
</tr>
<tr>
<td>Methionine, g/kg</td>
<td>0.36</td>
</tr>
</tbody>
</table>

1Vitamin and mineral premix provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 6.5 mg; vitamin B12, 0.2 mg; vitamin K3, 3.25 mg; biotin, 0.08 mg; folic acid, 1.5 mg; pantothenic acid, 12.5 mg; nicotinic acid, 45 mg; copper, 8 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; selenium, 0.15 mg; iodine, 0.35 mg.

2Nutrient level: ME was a calculated value, whereas other nutrient levels were measured values.
to determine the MDA concentration at a wavelength of 532 nm (Yang et al., 2010). The concentration of GSH was quantified at 405 nm by colorimetric detection of the glutathione reacted with 5,5′-dithio-bis-2-nitrobenzoic acid (Zhang et al., 2009). The activity of SOD was determined by the nitrite coloration method at 450 nm. The CAT activity was determined at 405 nm by the ammonium molybdate method. The GSH-Px activity was measured at 412 nm by quantifying the oxidation rate of reduced GSH to oxidized glutathione (Yang et al., 2010).

**Determination of Growth Hormone and Insulin-Like Growth Factor-1 Levels**

Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in serum were measured using corresponding ELISA kits (Liu et al., 2010; Sanders et al., 2010). Preparation of the monoclonal antibodies and coating of the microplate in the ELISA kits were performed by the Christina Research Laboratory at the University of California–Davis. Preparation of buffer solutions and protein standards was performed by the Bogoo Biological Technology Co. Ltd., Shanghai, China. This assay has high sensitivity and excellent specificity for the detection of chicken IGF-1 and GH. No significant cross-reactivity or interference between chicken IGF-1, GH, or any other analogs was observed. To assess intraassay precision, 3 samples of a known protein concentration were tested 20 times on the same plate, and the CV was calculated to be less than 8%. Intraassay precision was assessed using 20 assays of the 3 samples of known concentration, and the calculated CV was less than 10%. The microplates provided in these kits have been precoated with antibodies specific for chicken GH or IGF-1. To quantitatively determine the levels of GH or IGF-1 present in the samples, horseradish peroxidase-conjugated polyclonal antibodies specific for GH or IGF-1 were added to each well, followed by an enzyme-conjugated reagent. A standard curve was generated by plotting the optical density against the corresponding concentration of the standards and was subsequently used to determine the amounts of GH or IGF-1 in an unknown sample.

**Real-Time Quantitative PCR**

Total RNA from the spleen, thymus, and bursa tissue was isolated using TRIzol reagent (TaKaRa, Tokyo, Japan), and cDNA was synthesized from 1 μg of RNA with an MBI cDNA Synthesis Kit (MBI Fermentas, Hanover, MD) according to the manufacturer’s instructions. Based on the cloned complete sequences of Hsp27 (GenBank accession no. NM_205290.1), Hsp70 (no. EU747335.1), Hsp90 (no. DQ267486.1), and β-actin (no. L08165.1) from Gallus gallus domesticus, primer pairs (Hsp27/70/90 sense/Hsp27/70/90 antisense and β-actin sense/β-actin antisense) were designed for quantitative real-time PCR (Table 2). The β-actin gene was used as the housekeeping gene. All primers were synthesized and purified by Sangon Biotech (Shanghai) Co. Ltd. The PCR amplification reaction contained 12.5 μL of DreamTaq green 2× PCR Master Mix (Thermo Scientific, Rockford, IL), 1 μL of cDNA template, 1 μL of each of the upstream and downstream primers, and 10.5 μL of sterilized deionized water resulting in a total reaction volume of 25 μL. The amplification parameters of the thermocycler (BioRad, Richmond, CA) were a preheat period of 5 min at 94°C, 40 cycles of 30 s at 94°C, 40 s at 59°C, and 1 min at 72°C, and a final extension at 72°C for 10 min. Four microliters of PCR product were pipetted onto a 0.8% agarose gel and analyzed by gel electrophoresis. The real-time quantitative PCR reactions were performed in a 20-μL total reaction volume, which included 10 μL of SYBR Premix EX Taq master mix (TaKaRa), 0.4 μL of ROX Reference Dye (50×), 0.8 μL each of the forward and reverse primers (10 μM), 1 μL of cDNA template, and 7.8 μL of sterilized water. The PCR were carried out on an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). The thermal cycler parameters were as follows: 30 s at 95°C; 40 cycles for 5 s at 95°C, 31 s at 59°C, and an additional dissociation cycle of 15 s at 95°C, 1 min at 60°C, 15 s at 95°C, and 15 s at 60°C. The target gene expression was normalized to that of the selected reference gene, and the relative gene expression was determined using the 2−ΔΔCT method (Livak and Schmittgen, 2001). Threshold cycle values were obtained at the cycle number at which the gene was amplified beyond the designated threshold.

**Statistical Analysis**

All data from each cage were analyzed for a completely randomized design with cage as the experimental unit by the GLM procedure of SAS (SAS Institute Inc., Cary, NC). A single degree of freedom contrast was used to evaluate the effect of HT versus NT for birds on the basal diet without resveratrol. Orthogonal polynomial contrasts were also used to determine linear and quadratic responses of black-boned chickens to resveratrol levels in the HT environment. Statistical significance was determined at a probability level of 0.05.

**RESULTS**

**Performance**

The performance of birds during the 15-d experimental period is presented in Table 3. The daily FI (P < 0.01) and BWG (P < 0.001) were decreased in the HT groups compared with the NT groups; in contrast, F/G increased in the HT groups (P < 0.01). There was a quadratic response of BWG (P = 0.009) and F/G (P = 0.029) to dietary resveratrol supplementation for the birds subjected to HT (Table 3).
The effects of heat stress and resveratrol supplementation on the growth index of the bursa of Fabricius, thymus, and spleen are shown in Table 4. The index of the bursa of Fabricius and spleen were reduced \((P < 0.05)\) in HT-stressed black-boned chickens fed a diet without resveratrol compared with those subjected to the NT condition; however, the growth index of the thymus was not affected. Linear increases in the growth index of bursa of Fabricius were also observed upon HT treatment, which correlated with increased levels of supplemental resveratrol \((P < 0.05)\). The growth index of the thymus and spleen exhibited peak values for black-boned chickens supplied with the 400 mg of resveratrol/kg of diet upon HT treatment.

**Antioxidant Biomarkers**

Serum GSH levels and GSH-Px, SOD, and CAT activities were lower \((P < 0.001)\) in chickens subjected to HT compared with those reared under NT conditions. In contrast, the serum MDA levels were higher \((P < 0.001)\) in the HT groups compared with the NT groups (Table 5). The black-boned chickens exposed to HT displayed approximately 71% more serum MDA than of the NT chickens. A linear decrease \((P < 0.001)\) in serum MDA was observed when dietary resveratrol was added during HT treatment. The diets supplemented with different levels of resveratrol exhibited a linear increase \((P < 0.01)\) in serum GSH concentration and GSH-Px, SOD, and CAT activities. However, serum GSH-Px \((P < 0.001)\), SOD \((P < 0.01)\), and CAT \((P < 0.001)\) activities displayed a significant quadratic response in the presence of increasing concentrations of dietary resveratrol. These results suggest that resveratrol reduced \((P < 0.001)\) the concentration of lipid peroxidation products and increased \((P < 0.01)\) the activities of antioxidant enzymes in the serum of black-boned chickens exposed to heat stress (Table 5).

**GH and IGF-1**

The effects of heat exposure and resveratrol supplementation on serum GH and IGF-1 concentrations in black-boned chickens are shown in Figure 1. Serum concentrations of GH \((P < 0.001)\) and IGF-1 \((P < 0.01)\) significantly decreased in HT treated without resveratrol, compared with NT. However, dietary resveratrol supplementation linearly increased serum GH \((P < 0.01)\) and IGF-1 \((P < 0.001)\) concentrations of heat-stressed black-boned chickens. Serum IGF-1 concentrations showed significant quadratic response to dietary resveratrol supplement \((P = 0.002)\).

**Hsp27, Hsp70, and Hsp90 mRNA Expression Levels**

Differences in the induction of Hsp27, Hsp70, and Hsp90 mRNA expression were apparent among different organs (Figure 2). Both the bursa of Fabricius and spleen showed comparable Hsp27, Hsp70, and Hsp90 mRNA induction characteristics upon heat stress. The

<table>
<thead>
<tr>
<th>Primer name:1</th>
<th>Sequences of the primer pair</th>
<th>Fragment length, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp27 sense</td>
<td>5'-TAAGGATAACATCGTGAGATCA-3'</td>
<td>250</td>
</tr>
<tr>
<td>Hsp27 antisense</td>
<td>5'-CTACCTTCGCTTGCTTCT-3'</td>
<td></td>
</tr>
<tr>
<td>Hsp70 sense</td>
<td>5'-TGTTGCTCATCTACATTTGAG-3'</td>
<td>134</td>
</tr>
<tr>
<td>Hsp70 antisense</td>
<td>5'-GGCTTGCTACCTTGAAACCT-3'</td>
<td></td>
</tr>
<tr>
<td>Hsp90 sense</td>
<td>5'-GTCCTTCCTCTCCCTCCT-3'</td>
<td>233</td>
</tr>
<tr>
<td>Hsp90 antisense</td>
<td>5'-AGCCCAACAGAGAGATGACAC-3'</td>
<td></td>
</tr>
<tr>
<td>β-Actin sense</td>
<td>5'-CATCACCAGATCCCCACAAATA-3'</td>
<td>134</td>
</tr>
</tbody>
</table>

1Hsp = heat shock protein.

Table 3. Effect of heat stress and resveratrol supplementation on performance of black-boned chickens from age 42 to 57 d1

<table>
<thead>
<tr>
<th>Item</th>
<th>NT,2 added resveratrol, mg/kg</th>
<th>HT,2 added resveratrol, mg/kg</th>
<th>NT vs. HT at 0 mg of resveratrol/kg</th>
<th>Linear and quadratic effects of resveratrol under HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI,3 (g/bird per d)</td>
<td>43.5</td>
<td>38.5</td>
<td>40.8</td>
<td>42.3</td>
</tr>
<tr>
<td>BWG,3 (g/bird per d)</td>
<td>13.4</td>
<td>10.0</td>
<td>11.8</td>
<td>12.9</td>
</tr>
<tr>
<td>F/G,3</td>
<td>3.26</td>
<td>3.89</td>
<td>3.48</td>
<td>3.30</td>
</tr>
</tbody>
</table>

1Results are means of 6 replicate cages of 10 birds per cage.

2NT: normal temperature treatment (24 ± 2°C); HT: high temperature treatment (37 ± 2°C).

3FI: feed intake; BWG: BW gain; F/G: feed/gain ratio.
expression levels of *Hsp27*, *Hsp70*, and *Hsp90* mRNA in the bursa of Fabricius and spleen (*P* < 0.01), and *Hsp70* mRNA expression in the thymus were greater (*P* < 0.05) in black-boned chickens exposed to heat stress than the levels found in chickens exposed to normal environmental conditions. In contrast, *Hsp27*, *Hsp70*, and *Hsp90* mRNA expression in the bursa of Fabricius and spleen were inhibited in HT-treated birds fed with dietary resveratrol supplements. The expression of *Hsp70* and *Hsp90* mRNA in the bursa of Fabricius and spleen decreased in a linear manner (*P* < 0.001) as the supplemental resveratrol levels increased. The expression levels of *Hsp27*, *Hsp70*, and *Hsp90* in the spleens of HT-stressed chickens given 400 mg of resveratrol/kg of diet were lower than other resveratrol doses. These expression levels exhibited a significant quadratic response to increasing the dietary resveratrol supplemet levels (*P* = 0.004, *P* < 0.001, and *P* = 0.001, respectively).

In contrast, the thymus exhibited lower (*P* < 0.05) *Hsp27* and *Hsp90* mRNA induction in response to heat stress when compared with the other organs examined; however, *Hsp70* mRNA was expressed at a higher level in the thymus (Figure 2). The expression of *Hsp27* and *Hsp90* mRNA in the thymus was lower in black-boned chickens subjected to heat stress than in those reared in a normal environment (*P* < 0.01). Whereas an increase in *Hsp27* and *Hsp90* mRNA expression in the thymus correlated with increased doses of resveratrol supplements, the *Hsp70* mRNA of thymus exhibited an inverse correlation with resveratrol dose. Expression of *Hsp27* peaked, and *Hsp70* mRNA expression was at its lowest in the thymus of HT-treated black-boned chickens given 600 mg of resveratrol/kg of diet. These data were close to those obtained for chickens maintained under NT conditions.

**DISCUSSION**

Heat stress causes an imbalance in physiological status, a general reduction of the antioxidant system, and a reduction in the immunological function of chickens (Mujahid et al., 2007a; Panda et al., 2008). Plant polyphenols are a type of immune enhancer and antioxidant (Rice-Evans, 1995) that exert pleiotropic health benefits such as antibacterial, antiviral, anti-inflammatory, and anticancer effects (Duthie et al., 2000; Putics et al., 2008). It has been reported that resveratrol was one of the plant polyphenols (Yang et al., 2009) that acted as an anti-stress supplement in birds to alleviate tissue damage caused by heat stress (Sahin et al., 2012). The present investigation provides some evidence suggesting that dietary supplementation with resveratrol may improve the performance of black-boned chickens upon heat stress. The FI and BWG were significantly increased upon dietary resveratrol supplementation during heat stress, and the F/G ratio was decreased.

**Table 4. Effect of heat stress and resveratrol supplementation on index of bursa of Fabricius, thymus, and spleen in black-boned chickens**

<table>
<thead>
<tr>
<th>Immune organ growth index, mg/g</th>
<th>NT, $^2$ added resveratrol, mg/kg</th>
<th>HT, $^2$ added resveratrol, mg/kg</th>
<th>NT vs. HT at 0 mg of resveratrol/kg</th>
<th>Linear and quadratic effects of resveratrol under HT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>Bursa of Fabricius</td>
<td>1.06</td>
<td>0.57</td>
<td>0.72</td>
<td>0.76</td>
</tr>
<tr>
<td>Thymus</td>
<td>4.75</td>
<td>4.23</td>
<td>4.87</td>
<td>5.27</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.38</td>
<td>1.16</td>
<td>1.28</td>
<td>1.51</td>
</tr>
</tbody>
</table>

$^1$Results are the means of 6 replicate cages of 10 birds per cage.

$^2$NT: normal temperature treatment (24 ± 2°C); HT: high temperature treatment (37 ± 2°C).

$^3$MDA = malondialdehyde; GSH = glutathione; GSH-Px = GSH peroxidase; SOD = superoxide dismutase; CAT = catalase.

**Table 5. Effect of heat stress and resveratrol supplementation on the oxidative index and antioxidant enzyme activity in the serum of black-boned chickens**

<table>
<thead>
<tr>
<th>Oxidative and antioxidant index</th>
<th>NT, $^2$ added resveratrol, mg/kg</th>
<th>HT, $^2$ added resveratrol, mg/kg</th>
<th>NT vs. HT at 0 mg of resveratrol/kg</th>
<th>Linear and quadratic effects of resveratrol under HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA, $^3$ nmol/mL</td>
<td>0</td>
<td>0</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>3.37</td>
<td>5.78</td>
<td>4.85</td>
<td>4.24</td>
</tr>
<tr>
<td>GSH, $^3$ nmol/mL</td>
<td>1.85</td>
<td>0.80</td>
<td>1.09</td>
<td>1.42</td>
</tr>
<tr>
<td>GSH-Px, $^3$ 10$^6$ units/mL</td>
<td>23.45</td>
<td>12.68</td>
<td>18.57</td>
<td>21.58</td>
</tr>
<tr>
<td>SOD, $^3$ units/mL</td>
<td>224.12</td>
<td>172.18</td>
<td>195.92</td>
<td>210.38</td>
</tr>
<tr>
<td>CAT, $^3$ units/mL</td>
<td>60.95</td>
<td>21.73</td>
<td>48.45</td>
<td>52.36</td>
</tr>
</tbody>
</table>

$^1$Results are means of 6 replicate cages of 10 birds per cage.

$^2$NT: normal temperature treatment (24 ± 2°C); HT: high temperature treatment (37 ± 2°C).

$^3$MDA = malondialdehyde; GSH = glutathione; GSH-Px = GSH peroxidase; SOD = superoxide dismutase; CAT = catalase.
Exposure to chronic and irresistible heat stress may impair the balance between oxidative stress and antioxidant defense mechanisms via the depletion of enzymatic antioxidants and increased lipid peroxidation (Lin et al., 2000; Azad et al., 2010). The results of the present study also demonstrated a significant increase in MDA and lower levels of antioxidant enzymes in the HT treated group. These results are consistent with previous reports (Ramnath et al., 2008). Furthermore, resveratrol supplementation attenuated some of the heat stress-induced effects. Under thermo-neutral circumstances, resveratrol supplementation reduced the level of MDA and increased the activities of SOD, GSH-Px, and CAT (Sahin et al., 2010a, 2012). Several studies have shown that resveratrol can attenuate cellular processes associated with high temperature (Das, 2011; Sahin et al., 2012), UV radiation (Liu et al., 2011), lipopolysaccharide (Sebai et al., 2010), and ethanol-induced oxidative stress (Kasdallah-Grissa et al., 2007). Based on the results of this study, the improved antioxidant status of black-boned chickens observed in the resveratrol-supplemented groups could suggest that the resveratrol-induced effects were independent of the presence of heat stress, which is similar to data obtained with other antioxidants (Sahin et al., 2010b). Mujahid et al. (2007b) showed that excessive accumulation of MDA further inhibited the activities of antioxidant enzymes and accelerated the oxidative damage to proteins and DNA. Previous studies using resveratrol have suggested that it facilitates the inactivation and subsequent elimination of oxide precursors (Liu et al., 2011) and mobilizes the expression of antioxidant-related proteins (Sgambato et al., 2001), thus suggesting that it plays a protective role against oxidative stress. In this study, dietary supplementation of 400 mg of resveratrol/kg in animals subjected to HT exhibited a decrease in MDA concentrations and an elevation of the enzymatic activities of SOD, CAT, and GSH-Px to levels near those obtained from NT control animals. In addition, the diets supplemented with resveratrol increased GSH concentrations, which is an important mechanism for the prevention of damage induced by reactive oxygen species. There is evidence indicating that resveratrol scavenges reactive oxygen species and protects the organism from oxidative stress by improving the antioxidant capability to withstand thermal stress (Das, 2011). However, our data showed that resveratrol had a biphasic effect on the activation and inhibition of antioxidant enzymes and MDA formation and reduction; furthermore, resveratrol exhibited a dose-response pattern characterized by suitable-dose stimulation and high-dose inhibition.

Early studies suggested that an oxidative mechanism was involved in the growth-suppressive effects observed upon resveratrol treatment (Mujahid et al., 2005). Growth hormone and IGF are intimately related to the physiological processes of animal growth (Mitra et al., 1972; Sirotkin, 2010). This study provides experimental evidence that black-boned chicken with serum GH and IGF-1 deficiency exhibit impaired antioxidant status during chronic heat stress after 15 d. When the chickens were fed diets supplemented with resveratrol, serum GH and IGF-1 levels increased during heat stress, which appears to be an adaptive response to regulated growth and may also be an attempt to counteract heat stress-induced damage. This finding indicates that the level of IGF-1 stimulated by high GH levels is not necessarily higher upon resveratrol supplementation. Adding resveratrol to the diet seemed to increase the serum GH levels of black-boned chicken upon heat stress. Moreover, the corresponding levels of serum IGF-1 exhibited a complementary trend. These results suggest that resveratrol plays an important role in maintaining normal growth hormone levels in heat-stressed black-boned chickens.

![Figure 1](https://academic.oup.com/ps/article-abstract/93/1/54/1541430/6931547-541430)
Figure 2. Effects of heat temperature exposure and resveratrol level on heat shock protein (Hsp) 27, Hsp70, and Hsp90 mRNA expression in the bursa of Fabricius, thymus, and spleen of black-boned chickens. NT: normal temperature treatment (24 ± 2°C); HT: high temperature treatment (37 ± 2°C). Values are means ± SEM, n = 6. The Hsp27/70/90 mRNA expression of HT (resveratrol, 0 mg/kg) was different versus NT, *P < 0.05, **P < 0.01, ***P < 0.001 (NT vs. HT, without resveratrol). Linear contrasts of 0, 200, 400, and 600 mg of resveratrol in Hsp27 mRNA expression of the bursa of Fabricius, thymus, and spleen are expressed as P = 0.007, P = 0.004, and P = 0.001, respectively, and the quadratic contrast of resveratrol as P = 0.056, P = 0.198, and P < 0.001; linear contrasts of 0, 200, 400, and 600 mg of resveratrol in Hsp70 mRNA expression of the bursa of Fabricius, thymus, and spleen are expressed as P < 0.001, P = 0.005, and P < 0.001, and quadratic contrasts of resveratrol as P = 0.005, P = 0.686, and P < 0.001; linear contrasts of 0, 200, 400, and 600 mg of resveratrol in Hsp90 mRNA expression of bursa of Fabricius, thymus, and spleen are expressed as P < 0.001, P = 0.014, and P < 0.001, and the quadratic contrast of resveratrol as P = 0.011, P = 0.058, and P = 0.001, respectively.
The bursa of Fabricius, thymus, and spleen are important immunological organs in poultry. The thymus and bursa of Fabricius are central lymphoid organs (Cooper et al., 1966), and the spleen is the biggest peripheral immune organ in chickens (John, 1994), and is involved in cellular and humoral immunity. When broilers were subjected to heat stress, the relative weight of the spleen, thymus, and bursa of Fabricius decreased (Anwar et al., 2004); these results were in accordance with the development status of immunological organs in heat-stressed broilers reported by Bartlett and Smith (2003). In this study, the growth indices of the bursa of Fabricius and spleen in black-boned chickens decreased following 15 d of heat stress; however, the growth index of the thymus remained relatively constant. Das (2011) also observed that resveratrol was able to prevent body organ dysfunction induced by heat stress. Adding 400 mg of resveratrol/kg to the diet of thermal-stressed black-boned chickens can significantly increase the indices of the bursa of Fabricius, thymus, and spleen. This result implies that resveratrol was able to promote the growth of immune organs and counteract immune organ dysplasia caused by heat stress in black-boned chickens.

Several investigations have reported that heat stress increases the synthesis of the heat shock proteins Hsp27, Hsp70, and Hsp90, which are also constitutively expressed and play an essential protective role in maintaining the metabolic and structural integrity of the organ against stress-induced tissue injury (Mahmoud et al., 2004a; Yu et al., 2008). In this study, supplementation of resveratrol facilitated an induction of the endogenous antioxidant defense system, and these observations indicate that an improved antioxidant status could greatly attenuate heat stress-induced Hsp expression. This result is in agreement with the findings of Sahin et al. (2012), in which Hsp70 and Hsp90 expression was reduced in the liver tissue of heat-stressed quail. The present results demonstrate that the transcription of Hsp27, Hsp70, and Hsp90 increased in the bursa of Fabricius and spleen of black-boned chickens exposed to heat stress for 15 d. It was hypothesized that a high rate of Hsp translational activity in the bursa of Fabricius and spleen may be the result of heat stress increasing the workload of these immune organs by resisting oxidative damage. However, dietary supplementation with resveratrol decreased the expression of Hsp27, Hsp70, and Hsp90 genes in the bursa of Fabricius and spleen, a result that is consistent with the Hsp mRNA response to high temperatures and the correlation of these Hsp mRNA levels with tissue damage resulting from exposure to high temperature. The current study suggests that the diet supplemented with 400 mg of resveratrol/kg maintained Hsp27, Hsp70, and Hsp90 mRNA levels in the spleen of heat-stressed chickens to levels approximately in line with chickens that were subjected to NT conditions. During chronic and severe heat stress, the expression of Hsp27 and Hsp90 mRNA in the thymus of black-boned chickens were inhibited compared with the NT groups. This observation may be associated with the relatively high levels of these constitutively expressed Hsp27 and Hsp90 proteins found in other organs (Mahmoud et al., 2004b). This sequence of events could weaken Hsp expression in certain immune organs and reduce the stress protection capability of animals subjected to long-term heat stress. Nevertheless, resveratrol induced the expression of Hsp27 and Hsp90 mRNA in thymus and mobilized and activated the protective mechanisms of the immune organ. Natural polyphenolic compounds, such as resveratrol, feature overlapping properties that have elicited self-maintenance and protective mechanisms in animals (Sgambato et al., 2001; Murias et al., 2005; Putics et al., 2008). We speculate that this mechanism might be tissue specific and that the different levels of resveratrol and the different Hsp mRNA expression patterns may be organ specific. However, these mechanisms could be modulated by the metabolic requirements of the tissue.

In conclusion, resveratrol supplementation regulated the immune organ response to heat stress in black-boned chickens. The regulation occurred through the free radical scavenging capacity of the enzymatic and nonenzymatic antioxidant system and the induction of heat shock proteins in the immune organs.

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