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Berberis vulgaris root extract alleviates the adverse effects of heat stress via modulating hepatic nuclear transcription factors in quails

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Abstract

To evaluate the action mode of *Berberis vulgaris* root extract in the alleviation of oxidative stress, female Japanese quails (*n* 180, aged 5 weeks) were reared, either at 22°C for 24 h/d (thermoneutral, TN) or 34°C for 8 h/d (heat stress, HS), and fed one of three diets: diets containing 0, 100 or 200 mg of *B. vulgaris* root extract per kg for 12 weeks. Exposure to HS depressed feed intake by 8·5 % and egg production by 12·1 %, increased hepatic malondialdehyde (MDA) level by 98·0 % and decreased hepatic superoxide dismutase, catalase and glutathione peroxidase activities by 23·5, 35·4 and 55·7 %, respectively ($P< 0·001$ for all). There were also aggravations in expressions of hepatic NF-κB and heat-shock protein 70 (HSP70) by 42 and 43 %, respectively and suppressions in expressions of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and haeme-oxygenase 1 (HO-1) by 57 and 61 %, respectively, in heat-stressed quails ($P< 0·001$ for all). As supplemental *B. vulgaris* extract increased, there were linear increases in performance parameters, activities of antioxidant enzymes and hepatic Nrf2 and HO-1 expressions ($P< 0·001$ for all) and linear decreases in hepatic MDA level and NF-κB and HSP70 expressions at a greater extent in quails reared under TN condition and those reared under HS condition. In conclusion, dietary supplementation of *B. vulgaris* root extract to quails reduces the detrimental effects of oxidative stress and lipid peroxidation resulting from HS via activating the host defence system at the cellular level.

Key words: ***Berberis vulgaris*: Oxidative stress: Biomarkers: Defence system: Nuclear transcription factors: Heat stress**

Heat stress (HS) adversely affects survival[1], performance[2,3] and product quality[3] in poultry. Moreover, HS causes oxidative stress, reflected by an increased production of reactive oxygen molecules[4] and decreased concentrations of serum vitamins[5] and minerals[6] that play a role in the defence system. Oxidative stress impairs cell membrane and mitochondrial integrity[7] and causes cell damage through lipid peroxidation[4], which can be minimised by supplementation of antioxidant vitamins[8,9] or natural substances that possess antioxidant potential[10,11].

Berberis vulgaris L. (Barberry L. family Berberidaceae) is grown in Europe and Asia and its various parts (i.e. root, bark, leaf and fruit) are used in traditional medicine[12]. *B. vulgaris* extract exerts numerous biological effects that are pertinent to human medicine, which include antioxidant[13], anti-inflammatory[14,15], antimicrobial[16], antipyretic[12], antipruritic[12], antiurolithic[17], anticonvulsant[18] and antiarrhythmic[19] activities. The antioxidant effect of *B. vulgaris* extract is attributed to possessing high reductive powers to quench singlet molecular oxygen and peroxyl radicals[13]. The root of *B. vulgaris* is usually used for treating a variety of ailments such as diabetes, stomach, liver and kidney discomfort[16,20]. The predominant active compound in the plant is berberine, one of isoquinoline alkaloids[12,21].

Transcription factors are key cellular components that control gene expression in response to biological and environmental stimuli[22]. NF-κB is a pleiotropic transcription factor present in almost all cell types, responsible for controlling

Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; HO-1, haeme-oxygenase 1; HS, heat stress; HSP, heat shock protein; MDA, malondialdehyde; Nrf2, nuclear factor (erythroid-derived 2)-like 2; SOD, superoxide dismutase; TN, thermoneutral.

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DNA transcription and involved in cellular responses to a number of stimuli including free radicals[23]. This complex protein acts as the first responder because it is normally present in the cytosol in an inactive form and enters the nucleus in response to a stimulus in order to activate the expression of specific genes[23,24]. Heat shock proteins (HSP), known as stress proteins, are a group of proteins that are present in all cells and expressed at high levels when cells are exposed to high or low temperature or other stressors[25,26]. HSP play roles in protein folding and unfolding, protein assembling and disassembling and protein translocation[27,28].

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), another transcription factor, is known as a master regulator of the antioxidant response. It is bound to another protein called Kelch-like ECH (eroyl CoA hydratase)-associated protein 1 in the cytosol[29]. Disruption of cysteine residues in Kelch-like ECH-associated protein 1 due to oxidative stress results in accumulation of Nrf2 in the cytosol[30]. Unbound Nrf2 is then translocated into the nucleus, where its binds to the antioxidant response element in the promoter region of many antioxidative genes to initiate their transcriptions[31].

Recently, active components from herbal plants have been explored as possible antioxidants in poultry[32]. In a previous experiment, Kermanshahi & Riasi[33] demonstrated that the addition of dried *B. vulgaris* fruit to the diet of laying hens improved some blood and egg quality parameters that may have merit for the animal's well-being. Antioxidants are shown to modulate the transcription system[34,35]. However, the role of *B. vulgaris* root extract as an antioxidant against HS in poultry has not been investigated. The present experiment was performed to elucidate the mechanism by which supplemental *B. vulgaris* root extract alleviates performance and oxidative stress in Japanese quails (*Coturnix coturnix japonica*) exposed to HS.

Materials and methods

Plant material and extraction

The plant samples (root of *B. vulgaris*) were collected during the spring season of 2009 from a forest near Białystok, Poland. After crushing and drying, the samples (50 g) were subjected to extraction with water and methanol in Soxhlet apparatus for 32 h. The extract was concentrated to dryness under vacuum at 40°C. The extraction efficiency was 14·9 (SD 0·6) and 10·5 (SD 0·7) % (w/w), respectively, with water and methanol.

GC/MS analysis conditions

B. vulgaris root extract was assayed using a Perkin Elmer Clarus 680 gas chromatograph (Perkin Elmer Inc.) with a Clarus 600S mass spectrometer (Perkin Elmer Inc.). The separation of the analyte was performed on an Elite 5MS 30 m/0·25 mm column (Perkin Elmer Inc.) at the programmed thermostat temperature of −80°C at start-up, followed by increments of 5°C/min up to 320°C and isothermally for 22 min. The total time of analysis was 70 min. The injector temperature was 220°C and the flow of the carrier gas 1 ml/min. A split of 20 ml/min was applied. Electron ionisation with 70 eV was used in the spectrometer. The temperatures of the transfer line and the ion source were 240 and 200°C, respectively. The retrieved signals were identified using the National Institute of Standards and Technology mass spectra library. Signals with height three times larger than the background noises were assessed. Due to low content, berberine was identified using the selected ion research method for ions characteristic with masses of 339–164 and 337–321 m/s.

Evaluation of antioxidant activity and total phenolic content

Antioxidant activity of *B. vulgaris* root extract was determined as described by Velázquez *et al.*[36]. Briefly, 1·5 ml of a solution of 2,2-diphenyl-1-picrylhydrazyl were added to 0·75 ml of various concentrations of each sample solution ranging from 3·9 to 500 μg/ml. The solution of 2,2-diphenyl-1-picrylhydrazyl in methanol (20 mg/l) was prepared daily before UV measurements. The mixtures were kept in the dark for 15 min at room temperature and the decrease in absorbance was measured at 517 nm against a blank consisting of a 1·5 ml of methanol and 0·75 ml of extract solution. Quercetin and gallic acid were used as positive controls. These were converted to percentage 2,2-diphenyl-1-picrylhydrazyl radical scavenging[37]. The half-maximal inhibitory concentration value of each extract was determined graphically and all tests were performed in triplicate. A lower half-maximal inhibitory concentration value indicates stronger antioxidant activity.

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method; the results were expressed as mg of gallic acid equivalents per g of extract[38].

Animals, diets and experimental design

A total of 180, 5-week-old female Japanese quails (*C. coturnix japonica*) were used in accordance with animal welfare regulations at the Veterinary Control and Research Institute of Elazig, Turkey. All procedures involving birds were approved by the Institutional Animal Care and Use Committee at the Institute of Veterinary Research, Elazig, Turkey (approval code: 2011/5-1). The quails were kept in temperature-controlled rooms at either 22°C for 24 h/d (thermoneutral, TN) or 34°C for 8 h (09.00 and 17.00 hours) followed by 22°C for 16 h/d (HS) during the experimental period. The quails were then fed one of three diets: basal diet and basal diet supplemented with 100 or 200 mg *B. vulgaris* root extract per kg diet. Chemical composition of *B. vulgaris* root extract is shown in Table 1. The diets (Table 2) were mixed weekly in batches and stored in black plastic containers at 4°C to avoid photo-oxidation in airtight containers.

All quails were hatched from a large group of the parent stock that were of identical age. Each of the 2 × 3 factorially arranged groups was replicated in ten cages (20 × 20 cm^2 dimension), each consisting of three quails. During the experimental period (12 weeks), the birds were subjected to a 16 h light–8 h dark cycle and offered feed and water *ad libitum*.

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Table 1. Chemical compositions of *Berberis vulgaris* root extract with water and methanol*

Extraction with water			Extraction with methanol		
Compound	RT	%	Compound	RT	%
Glycerine	6·71	2·72	2-Hydroxy-gamma-butyrolactone	7·34	2·11
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	7·28	0·31	Pentanal	10·27	0·60
2-Hydroxy-gamma-butyrolactone	7·46	0·54	1,2-Methyl-4-hydroxyacetophenone	16·14	0·42
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	8·98	1·52	Sucrose	19·08	0·35
1,3,5-Triazine-2,4,6-triamine	9·53	1·27	1-Dodecanol	20·06	0·23
Maltol	10·87	2·27	Dodecanoic acid	22·05	4·94
4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	11·74	27·94	Lauryl acrylate	25·23	0·60
1,2-Benzenediol	12·88	2·24	Tetradecanoic acid	26·65	3·65
5-Hydroxymethylfurfural	13·88	10·28	Tetradecanal	28·02	0·25
4-Hydroxy-3-methylacetophenone	16·42	5·79	Ferulic acid methyl ester	28·82	0·83
Unknown	18·82	1·95	2-Nonadecanone	29·75	0·23
Sucrose	19·81	14·01	Hexadecanoid acid methyl ester	30·18	2·17
Unknown	21·20	1·78	*N*-Hexadecanoid acid	30·92	38·23
Unknown	21·86	0·55	Linoleic acid methyl ester	33·53	0·46
Homovanillyl alcohol	22·52	0·28	Octadecanoic acid methyl ester	34·06	5·41
Ethanone,1-(3,4-dimethoxyphenyl)-	22·87	0·72	Octadecanoic acid	34·75	24·35
Unknown	23·50	2·81	Hexadecanamide	35·21	5·12
n-Octyl propyl ether	24·18	4·20	Octadecanamide	38·8	1·62
Unknown	25·30	0·69	Cholest-5-en-3-ol (3α)	50·6	0·44
L-Glucose	25·46	0·49	Dihydroberberine	52·89	0·03
2-Amino-3-hydroxypyridine	27·50	2·70	γ-Sitosterol	54·41	1·93
4-Methoxyphenyl 4 propylcylohexanecarboxylate	29·21	0·91	Pentacosane	58·12	3·22
Octadecanal	29·98	0·64	Pentatricontane	63·17	2·81
N-Hexadecanoic acid	31·36	10·44			
Octadecanoic acid, ethenyl ester	37·88	0·31			
13-Docosenoic acid, methyl ester	38·20	1·32			
2H-pyran-2-one, tetrahydro-6-tridecyl	38·82	0·52			
Octadecanamide	39·31	0·15			
Tetrahydroxyberberine	44·93	0·65			

RT, retention time (min).
* Total area was 1 099 497 784 and 716 735 822 in extraction with water and methanol, respectively.

Sample and data collection

Feed intake was measured weekly and egg production was recorded daily during the experimental period. At the end of the experiment, one bird from each cage (ten birds per group) was killed by cervical dislocation. The liver was removed and chopped into small pieces on ice for determination of the oxidative stress biomarkers, including levels of malondialdehyde (MDA) and antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px)) and expressions of hepatic nuclear transcription proteins (NF-κB, HSP70, Nrf2 and haeme-oxygenase 1 (HO-1)).

Laboratory analyses

Lipid peroxidation and antioxidant enzymes. A 10 % (w/v) liver homogenate was prepared in 10 mM-phosphate buffer (pH 7·4). The homogenate was centrifuged at 13 000 ***g*** for 10 min at 4°C. The supernatant was collected and stored at −80°C. The concentration of MDA, an index of lipid peroxidation and oxidative stress, was measured[39] using the fully automatic HPLC (Shimadzu) equipped with a pump (LC-20AD), an ultraviolet–visible detector (SPD-20A), an inertsil ODS-3 C_{18} column (250 × 4·6 mm, 5 m), a column oven (CTO-10ASVP), an autosampler (SIL-20A), a degasser unit (DGU-20A5) and a computer system with LC solution Software (Shimadzu). Total SOD activity was attained based on the amount of enzyme required to inhibit the rate of formazan dye formation by 50 %[40]. CAT activity was estimated based on the amount of enzyme required to decompose 1 mmol of H_2O_2 to water and oxygen[41]. GSH-Px activity

Table 2. Ingredient and nutrient composition of the basal diet*

Ingredient	Amount (g/kg)
Maize	537·6
Soyabean meal, 44 % crude protein	292·7
Soya oil	48·5
Salt	3·1
DL-Met	2·0
Limestone	95·0
Dicalcium phosphate	17·6
Vitamin and mineral premix†	3·5
Nutrients (g/kg)	
Metabolisable energy (kJ/kg)‡	11 850
Crude protein	179·5
Ca	39·6
P	6·3
Met‡	4·2
Lys‡	10·5

* *Berberis vulgaris* root extract (0, 200 or 400 mg *B. vulgaris* per kg diet) was added to the basal diet at the expense of maize.
† Per kg contained: vitamin A, 8000 IU; vitamin D_3, 3000 IU; vitamin E, 25 IU; menadione, 1·5 mg; vitamin B_{12}, 0·02 mg; biotin, 0·1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg. Cu (copper sulphate), 10·00 mg; iodine (ethylenediamine dihydriodide), 1·00 mg; Fe (ferrous sulphate monohydrate), 50·00 mg; Mn (manganese sulphate monohydrate), 60·00 mg; and Zn (zinc sulphate monohydrate), 60·00 mg; Se (sodium selenite), 0·42 mg.
‡ Calculated value according to tabular values listed for the feed ingredients[44].

assessment was based on the amount of enzyme required to oxidise 1 mmol NADPH[42]. The enzyme activities were expressed as U/mg protein.

Western blot analyses. Accurately weighed liver tissue was homogenised in 1:10 (w/v) ratio in 10 mM-Tris–HCl buffer (pH 7·4) containing 0·1 mM-NaCl, 0·1 mM-phenylmethylsulphonyl fluoride and 5 μM-soyabean (soluble powder; Sigma) as trypsin inhibitor. Tissue homogenate was centrifuged at 15 000 ***g*** at 4°C for 30 min and the supernatant was transferred into fresh tubes. SDS-PAGE sample buffer containing 2 % β-mercaptoethanol was added to the supernatant. Equal amounts of protein (20 μg) were electrophoresed and subsequently transferred to nitrocellulose membrane (Schleicher and Schuell, Inc.). Nitrocellulose blots were washed twice for 5 min in PBS and blocked with 1 % bovine serum albumin in PBS for 1 h prior to the application of primary antibody. Chicken antibodies against NF-κB, HSP70, Nrf2 and HO-1 (Abcam) were diluted (1:1000) in the same buffer containing 0·05 % Tween 20. The nitrocellulose membrane was incubated overnight at 4°C with protein antibody. The blots were washed and incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Abcam). Specific binding was detected using diaminobenzidine and H_2O_2 as substrates. Protein loading was controlled using a monoclonal mouse antibody against β-actin antibody (Sigma). Samples were analysed in quadruplicates and protein levels were determined densitometrically using an image analysis system (Image J; National Institutes of Health).

Dietary nutrients. The diets were analysed for crude protein (no. 988·05), Ca (no. 968·08) and P (no. 965·17) according to the procedures described by the Association of Official Analytical Chemists[43]. Energy and amino acid (methionine and lysine) contents were calculated from tabular values listed for the feedstuffs[44].

Statistical analysis

Data on performance parameters, oxidative stress biomarkers and proteins were analysed by two-way ANOVA using the PROC GLM procedure[45]. The linear model to test the effect of treatments on response variables was as follows: $y_{ijk} = \mu + E_i + S_j + (E \times S)_{ij} + e_{ijk}$, where y = response variable, μ = population mean, E = environmental temperature, S = *B. vulgaris* root extract supplementation and e = residual error (N (σ, μ; 0, 1)). The model also included polynomial contrast to determine changes in response variables as supplemental *B. vulgaris* root extract level was increased. Correlations among performance parameters, oxidative stress biomarkers and nuclear proteins were determined using the CORR procedure[45]. Statistical significance was considered at $P \leq 0·05$.

Results

Composition of *Berberis vulgaris* and antioxidant activity

Table 1 lists twenty-three compounds detected in *B. vulgaris* root extract (>0·05 % of total ion current). *B. vulgaris* root extract antioxidant activity value was 60·24 (SD 6·27) %. Total phenolic content was 8·92 (SD 0·55) mg gallic acid equivalents per g extract.

Performance

Table 3 summarises performance variables. Quails exposed to HS consumed less feed (27·6 *v.* 30·2 g/d) and produced less egg (81·6 *v.* 92·8 %) than those reared under the TN environment ($P < 0·0001$ for both). Overall, there were 3·1 and 4·9 % increases in feed intake and egg production in response to increasing dietary *B. vulgaris* root extract supplementation (linear effect; $P < 0·0001$ for both). These increases were greater in the HS environment than in the TN environment (environmental temperature × *B. vulgaris* root extract supplementation level interaction effect, $P < 0·001$ for both).

Hepatic malondialdehyde and antioxidant enzymes

Heat-stressed quails had higher hepatic MDA level (2·70 *v.* 1·36 nmol/g) and lower hepatic SOD (126 *v.* 175), CAT (28 *v.* 49) and GSH-Px (10 *v.* 18) activities (U/mg protein) than control quails ($P < 0·001$ for all). There was a linear decrease in level of MDA (by −25·5 %) and increases in activities of SOD, CAT and GSH-Px (by 23·5, 35·4 and 55·7 %, respectively) as supplemental *B. vulgaris* root extract level in the diet increased ($P < 0·001$ for all). Decrease in hepatic MDA level and increases in hepatic SOD, CAT and GHS-Px activities in response to increasing *B. vulgaris* root extract supplementation were more notable in the HS environment than in the TN environment (environmental temperature × *B. vulgaris* root extract level interaction effects; $P < 0·001$ for all; Table 4).

Table 3. The effects of *Berberis vulgaris* root extract supplementation (BV) (mg/kg) on performance of quails exposed to HS

	ET									
	TN			HS				Statistical significance ($P > F$)		
Variable	0	200	400	0	200	400	SEM	ET	BV	ET × BV
Feed intake (g/d)	30·2	30·5	29·8	26·6	27·6	28·7	0·2	0·0001	0·0001	0·001
Egg production (%)	92·8	92·5	93·1	77·7	81·3	85·7	1·3	0·0001	0·0001	0·001

ET, environmental temperature; TN, thermoneutral environment (temperature-controlled rooms at 22°C for 24 h/d); HS, heat stress environment (34°C for 8 h between 09.00 and 17.00 hours followed by 22°C for 16 h/d).

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Hepatic nuclear transcription factors

Expressions of hepatic NF-κB (161·6 *v.* 94·1 %) and HSP70 (161·2 *v.* 91·4 %) were greater, whereas that of hepatic Nrf2 (72·1 *v.* 113·1 %) and HO-1 (69·8 *v.* 112·8 %) were lower in the heat-stressed quails than the control quails ($P<0·001$ for all). With increasing dietary *B. vulgaris* root extract supplementation, the mean expressions of hepatic NF-κB (by −22·7 %) and HSP70 (by −26·6 %) decreased linearly ($P<0·001$ for both), whereas the mean expressions of hepatic Nrf2 (by 56·0 %) and HO-1 (by 38·0 %) increased linearly ($P<0·001$ for both). Decreases in hepatic NF-κB and HSP70 levels ($P<0·001$ for both) and increases in hepatic Nrf2 and HO-1 levels ($P<0·001$ for both) in response to increasing supplemental *B. vulgaris* root extract level were greater in the TN environment than in the HS environment (environmental temperature × *B. vulgaris* root extract level interaction effects; Table 4).

Correlations among oxidative stress biomarkers and protein levels

All response variables were autocorrelated ($P<0·001$; Table 5). There were positive correlations between egg production and activities of antioxidant enzymes and negative correlation between egg production and hepatic MDA level. Hepatic MDA level was negatively correlated with activities of antioxidant enzymes and expressions of Nrf2 and HO-1 and positively correlated with expressions of NF-κB and HSP70. Moreover, activities of antioxidant enzymes were negatively correlated with expressions of NF-κB and HSP70 and positively correlated with expressions of Nrf2 and HO-1.

Discussion

HS compromises performance and productivity through reducing feed intake, while decreasing growth rate, egg production, egg quality and feed efficiency, which lead to economic losses in poultry. HS also leads to oxidative stress associated with a reduced antioxidant status in the bird *in vivo,* as reflected by increased oxidative damage and lowered plasma concentrations of antioxidants. In agreement with the literature, HS caused depressions in feed intake and egg production (Table 3). Moreover, supplemental *B. vulgaris* extract increased feed intake and egg production at a greater extent in heat-stressed quails than control quails (Table 3). These positive impacts of *B. vulgaris* root supplementation on performance are in agreement with a previous study[47,47]. Moreover, Kermanshahi & Riasi[33] reported that dried *B. vulgaris* fruit significantly improved haematocrit value and HDL-cholesterol in laying hens.

Cell damage occurring due to oxidative stress results in lipid peroxidation[47,48]. This is reflected by elevated hepatic MDA level (Table 4). *B. vulgaris* root extract is a free radical scavenger and protects membrane stability[13,49]. Several studies reported that *B. vulgaris* root extract possesses antioxidant activity at different degrees in different extraction solvents[37,50]. Hanachi & Golkho[50] reported that the antioxidant activity of ethanol extract was the highest (27·26 %), followed by extraction with butylated hydroxytoluene (20·29 %), methanol extract (16·80 %), vitamin E (6·68 %) and water (6·53 %). Moreover, because the extract is a pool of many named and unnamed compounds, it is always difficult to conclude the responsibility of each chemical constituent.

Living organisms facilitate the antioxidant defence system through increasing activities of detoxifying enzymes (SOD, CAT and GSH-Px (Table 4)) in order to reduce harmful effects of reactive oxygen molecules produced due to HS[48]. *B. vulgaris* root extract supplementation elevates the activity of SOD, CAT and GSH-Px (Table 4), probably due to its antioxidant nature. Due to limitation of literature regarding the effects of *B. vulgaris* root extract on the antioxidant enzymes, the present enzyme data are not comparable. However, its antioxidant effects were postulated to be linked to cytotoxicity in human liver cell lines[51]. In the present study, *B. vulgaris* root extract's antioxidant activity was 60·24 (SD 6·27) %, which is in agreement with a report by Zovko-Končić

Table 4. The effects of *Berberis vulgaris* root extract supplementation (BV) (mg/kg) on hepatic oxidative stress biomarkers and expressions of hepatic nuclear transcription factors in quails exposed to heat shock

	ET							Statistical significance ($P>F$)		
	TN			HS						
Variable*	0	200	400	0	200	400	SEM	ET	BV	ET × BV
Oxidative stress biomarkers										
MDA (nmol/g)	1·59	1·32	1·18	3·12	2·65	2·33	0·09	0·001	0·001	0·001
SOD (U/mg protein)	162	178	185	106	127	146	3·4	0·001	0·001	0·001
CAT (U/mg protein)	42	51	55	23	28	33	1·5	0·001	0·001	0·001
GSH-Px (U/mg protein)	14·2	18·5	21·9	7·9	9·8	12·5	1·03	0·001	0·001	0·001
Nuclear transcription factors										
NF-κB	100	95·4	86·8	186·5	163·6	134·6	4·5	0·001	0·001	0·001
HSP70	100	91·9	82·3	195·5	153·4	134·7	5·1	0·001	0·001	0·001
Nrf2	100	112·3	126·9	42·6	78·3	95·5	3·8	0·001	0·001	0·001
HO-1	100	114·9	123·6	50·2	75·4	83·7	5·0	0·001	0·001	0·001

ET, environment temperature; TN, thermoneutral environment (temperature-controlled rooms at 22°C for 24 h/d); HS, heat stress environment (34°C for 8 h between 09.00 and 17.00 hours followed by 22°C for 16 h/d); MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; HSP70, heat shock protein 70; Nrf2, nuclear factor (erythroid-derived 2)-like 2; HO-1, haeme oxygenase-1.

* Expressions of nuclear transcription factors are percentage of control (quails reared under the TN condition and not received *B. vulgaris* root extract).

Table 5. Pearson's correlation coefficients (*r*) among performance variables, hepatic oxidative stress biomarkers and hepatic nuclear transcription factors*

Variables	EP	MDA	SOD	CAT	GSH-Px	NF-κB	HSP70	Nrf2	HO-1
FI (g/d)	0·83	−0·91	0·82	0·76	0·73	−0·87	0·82	0·78	0·82
EP (%)		−0·78	0·81	0·83	0·75	−0·83	0·83	0·75	0·74
MDA (nmol/g)			−0·82	−0·86	−0·83	0·91	0·92	−0·81	−0·91
SOD (U/mg protein)				0·88	0·85	−0·94	−0·86	0·86	0·84
CAT (U/mg protein)					0·92	−0·85	−0·76	0·75	0·87
GSH-Px (U/mg protein)						−0·89	0·86	0·86	0·90
NF-κB							0·93	−0·86	−0·93
HSP70								−0·90	−0·85
Nrf2									0·95

FI, feed intake; EP, egg production; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; HSP70, heat shock protein 70; Nrf2, nuclear factor (erythroid-derived 2)-like 2; HO-1, haeme oxygenase-1.
* $P < 0.001$ for correlation coefficients for all paired variables.

et al.[13]. It was shown that total phenolic content of *B. vulgaris* increases the antioxidant activity[37]. In the present study, total phenolic content of *B. vulgaris* root extract was 8·92 (SD 0·55) %. Despite not determined in the present study, other studies showed that antioxidant effects of *B. vulgaris* root extract could be related to its vitamin C, malic acid and tannin contents[50]. In oxidative stress induced in mice by CCl_4 intoxication, Domitrović *et al.*[52] reported an increase in MDA level and a decrease in SOD activity, which were ameliorated by *B. vulgaris* root extract. This was attributed to berberine, an isoquinoline alkaloid found in *B. vulgaris* root extract. Similarly, the present study demonstrated aggravated lipid peroxidation and suppressed activities of oxidative enzymes in heat-stressed quails, which were alleviated by *B. vulgaris* root extract supplementation in a dose–response manner (Table 4).

NF-κB and Nrf2 are redox-sensitive transcription factors[53] and play a role in induction of phase II detoxifying/antioxidant defence mechanisms to cope with oxidative stress through enhancing the expression of a number of enzymes, such as NAD(P)H quinone oxidoreductase 1, glutamate cysteine ligase, HO-1, glutathione *S*-transferase and uricline diphosphate (UDP)-glucuronosyltransferase[54]. In addition, NF-κB is induced by various cell stress-associated stimuli, including oxidative stress. HS-induced oxidative stress increases production of reactive oxygen molecules that lead to the activation of various redox-sensitive cell signalling molecules such as NF-κB[55,56]. The literature on the NF-κB and Nrf2 pathway activity in response to dietary supplementation of *B. vulgaris* root extract in poultry is limited. In the present study, increased expression of NF-κB (Table 4) and decreased expression of Nrf2 (Table 4) in heat-stressed quails could be related to their activation[23,24] and translocation[30,57], respectively, to overcome oxidative stress induced by HS. In the present study, the levels of berberine were 0·03 %. Among several alkaloids of *B. vulgaris*, berberine was considered to be responsible for most of the biological activity, such as abolished acetaldehyde-induced NF-κB activity and cytokine production in a dose-dependent manner[58]. Liu *et al.*[59] also reported that the immunostaining of NF-κB was decreased and the reduced degradation of inhibitor of κB level was partially restored after berberine treatment in alloxan-induced diabetic mice. Increased berberine-increased HO-1 expression is mediated by Nrf2 activation[60], suggesting that *B. vulgaris* root extract acts as modifier of signal transduction pathways to elicit cytoprotective responses.

Several studies have shown that HS triggers expression of HSP70 induction[25,61]. To our knowledge, the present study is the first to evaluate the effect of the *B. vulgaris* root extract supplementation on HSP in the liver of heat-stressed Japanese quail. In the present study, the HSP70 expression was much lower in quails supplemented with *B. vulgaris* root extract than control quails (Table 4). Other studies have ascertained the protective role of antioxidants by suppressing HSP expressions in stressed birds[61,62].

In conclusion, *B. vulgaris* root extract supplementation compensated depression in performance variables (feed intake and egg production) in heat-stressed quails and alleviated oxidative stress, as reflected by lipid peroxidation and activity of antioxidant enzymes. Moreover, *B. vulgaris* root extract exerted antioxidant effects through inhibiting NF-κB and HSP70 expressions and activating Nrf2 and HO-1 expressions, which were activated and suppressed in the HS environment, respectively. Future studies should focus on elucidation of the effect of chemicals on the modulation of oxidative stress biomarkers and nuclear transcription proteins.

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