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Original article

Gene expression of heat shock proteins/factors (HSP60, HSP70, HSP90, HSF-1, HSF-3) and antioxidant enzyme activities in heat stressed broilers treated with vitamin C

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Abstract

In broiler chickens, the relationship between dietary supplementation of vitamin C and hepatic, cardiac and renal heat shock proteins (HSP60, HSP70 and HSP90), heat shock factors (HSF-1 and HSF-3) and enzymatic antioxidants requires further investigation. The current study aimed to investigate this relationship at cellular and molecular levels in a 42 days experiment. Two hundred, one-day-old broiler chicks (Ross 308) were allocated into four equal groups. Chicks in the first and third groups were thermo-neutral (TN; 22°C for 24 hours/day) and fed basal diet without or with vitamin C (1g/kg basal diet), respectively. Chicks in the second and fourth groups were heat stressed (HS; 34°C for 8 hours/day) and fed basal diet without or with vitamin C, respectively. Performance parameters were recorded throughout the experiment. Levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPX), Catalase (CAT) and gene expression of heat shock proteins (HSP60, 70 and 90) and heat shock factors (HSF 1 and 3) were analyzed in liver, heart and kidney tissues of the studied groups. Heat stress induced a negative impact on performance parameters, significant reduction in activities of all examined antioxidant enzymes and a significant up-regulation in heat shock proteins and factors genes in all studied tissues. Dietary supplementation of vitamin C corrected these parameters towards the normal control values. Conclusively, dietary supplementation of the examined dose of vitamin C was efficient at ameliorating the detrimental effects of heat stress on liver, heart and kidney tissues of broilers chickens at cellular and molecular levels.

Key words: ascorbic acid, tissues, chicken, peroxidation, biomarkers

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Introduction

Acute exposure of chickens to heat stress causes a depression in feed ingestion and growth performance resulting in high economic losses (Ismail et al. 2013, Ismail et al. 2015). Heat stress induced free radical production and decreased the antioxidant system creating oxidative stress and tissues damage (Akbarian et al. 2016). Alleviation of the negative effect of heat stress through cooling of the animal house is impractical and costly; therefore workers directed their efforts towards dietary manipulation. Vitamin C is not typically added to poultry diets; however, under a heat stress the metabolic need for vitamin C exceeds the synthesizing capacity of the birds (Asli et al. 2007) and supplemented ascorbic acid could significantly reduce heat stress and stimulate enzymatic antioxidants (Ismail et al. 2013, 2015). Oxidative stress is the main factor which activates heat shock proteins (HSPs; Ananthan et al. 1986) to protect against tissue damage (Hightower 1991). HSPs play an important role in maintaining the integrity of structural proteins and the folding-refolding of damaged proteins (Bukau and Horwich 1998, Kregel 2002). According to their molecular size, there are six main families of HSPs, namely, HSP100, HSP90, HSP70, HSP60, HSP40, and the small HSPs (Morimoto 1998, 2008). Of the many expressed HSPs, those with a molecular weight of approximately 70 kDa appear to be most closely associated with heat tolerance (Wang and Edens 1998). Heat shock factors (HSFs) are transcription factors that regulate the expression of heat shock proteins (Morimoto 1998, Pirkkala et al. 2001). Four heat shock factors are known to regulate the expression of HSPs (Morimoto 1998). HSF-1 acts as a classic stress-responsive factor, inducing transcriptional activity of heat shock genes in response to diverse forms of stress (Fujimoto and Nakai 2010). HSF-2 is not activated in response to classic stress stimuli (Eriksson et al. 2000). HSF-3 is a unique avian-specific HSF while HSF-4 is a mammalian HSF (Inouye et al. 2003). Among the three HSF genes expressed in avian cells, HSF1 and HSF3 are considered redundant heat shock responsive factors (Fujimoto and Nakai 2010). The studies reporting the effect of vitamin C on HSP regulation at molecular levels are contradictory. Some works concluded that the presence of antioxidant enzymes, particularly CAT and SOD (Omar and Pappolla 1993) or vitamin C (Mahmoud et al. 2003, 2004), ameliorated the heat stress and prevented the induction of HSPs in broilers. Another report (Chandrakant et al. 2012) concluded that ascorbic acid (150mg/kg) downregulated HSP70 in the jejunum and liver of heat stressed broilers. Other studies, however, (Kapakin et al. 2012) demonstrated that, vitamin C (L-Ascorbic acid) enhanced the secretion of HSP70 in the liver and kidney of heat stressed broiler chickens. Therefore, the relationship between dietary supplementation of vitamin C and hepatic, cardiac and renal heat shock proteins (HSP60, HSP70 and HSP90), heat shock factors (HSF-1 and HSF-3) and enzymatic antioxidants requires further investigation. Therefore, the objective of the current study was to investigate the impact of dietary supplementation of vitamin C on the gene expression of heat shock proteins/factors and the activities of antioxidant enzymes in heat stressed broilers.

Materials and Methods

Birds, feed additives and performance parameters

One-day old broiler chicks (Ross 308; 38 ± 2g body weight; n=200) were used in the current experiment (42 days) and allocated to 4 groups (50 chicks each). Each group consisted of 5 replicates (10 birds/replicate). The birds were housed in a closed system poultry house at the Research Station, King Faisal University, Al-Ahsa, Saudi Arabia. The birds were maintained as required by national guidelines and protocols and this study was approved by the University Scientific Research Ethics Committee, King Faisal University, Saudi Arabia (Project # 170111). The pens provided a floor area of 1.5m^2. The regular vaccination program applied to all birds. Birds were exposed to 22 h of light a day and 56% relative humidity (RH) during the experimental period (1-42 days). Chicks in the first group were thermo-neutral (TN; 22°C for 24 hours/day) and fed on a basal diet without any additives along the experimental period (42 days). Chicks in the second group were subjected to heat stress (HS; 34°C for 8 hours/day) and fed on a basal diet without any additives during the experimental period (42 days). Chicks in the third group (TN + Vitamin C) were thermo-neutral (22°C for 24 hours/day) and fed on a basal diet supplemented with Vitamin C (L-Ascorbic acid 99%; Sigma Aldrich Cat #A92902; 1g/kg basal diet; Ismail et al. 2013, 2015) during the experimental period (42 days). Chicks in the fourth group (HS + Vitamin C) were subjected to heat stress (34°C for 8 hours/day) and fed on a basal diet supplemented with Vitamin C (1g/kg basal diet) along the experimental period (42 days). HS birds were subjected to the same TN temperature (22°C) during the last 16 hours of the day during the experimental period (42 days). Commercial broiler feed (ARASCO, Saudi Arabia; starter code No. 24203; Finisher code No. 24402) was used as a basal diet (TN birds) throughout the experimental period. The feed was formulated from cereal, soybean meal, vegetable oil, vitamins and bio-

Table 1. Calculated analysis of basal diet.

Diet ingredients (g/kg)	Unit	Starter	Finisher
Metabolizable energy	Kcal/kg	2900	3000
Minimum Crude protein	%	21.0	18.5
Minimum Crude fat	%	2.5	3.0
Maximum fibre	%	3.0	3.5
Calcium	%	1.0	0.9
Available Phosphorus	%	0.42	0.4
Sodium	%	0.15	0.15
Lysin	%	1.20	1.0
Methionine	%	0.50	0.45
Methionine+ cystine	%	0.85	0.80
Vitamin A	IU/kg	12000	12000
Vitamin D	IU/kg	3000	3000
Vitamin E	mg/kg	60	60
Vitamin C	mg/kg	100	100
Vitamin K	mg/kg	4.0	4.0
Vitamin B_1	mg/kg	3.0	3.0
Vitamin B_2	mg/kg	8.0	8.0
Vitamin B_6	mg/kg	5.0	5.0
Vitamin B_{12}	mg/kg	0.03	0.03
Niacin	mg/kg	40.0	40.0
Pantothenic acid	mg/kg	15	15
Folic acid	mg/kg	2.0	2.0
Biotin	mg/kg	0.2	0.2
Choline	mg/kg	900	900
Cobalt	mg/kg	0.5	0.5
Copper	mg/kg	8.0	8.0
Iodine	mg/kg	2.0	2.0
Iron	mg/kg	35.0	35.0
Manganese	mg/kg	90	90
Selenium	mg/kg	0.2	0.2
Zinc	mg/kg	70	70
Antioxidant	mg/kg	125	125

plex minerals. Coccidiostat (Avatec/Cygro) was added only to the starter diet. The calculated analysis of the commercial diet is shown in Table 1 (Ismail et al. 2013, 2015). Feed intake, body weight gain, feed conversion ratio and mortality percentage were recorded throughout the experiment (Table 2). Feed conversion ratio (kg feed/kg gain) was calculated by dividing feed intake with body weight gain (Yi et al. 2018).

Sample collection

At the end of the experiment (42 days), 3 chicks from each replicate (15 birds/group) were humanely euthanatized and liver, heart and kidney tissues were collected. Each tissue was divided into two portions. The first portions of different tissues were homogenized and the supernatant of each relevant tissue homogenate kept frozen at -80ºC until used for biochemical analysis of the concentration of MDA and the activities of antioxidant enzymes SOD, GST, GPX and CAT. The second portions of collected tissues were stored frozen at -80°C until used for analysis of gene expression of heat shock proteins (HSP60, 70 and 90) and heat shock factors (HSF 1 and 3). Euthanasia was performed after sodium pentobarbital anesthesia (20-30 mg/kg; Overmyer et al. 2015). Sodium pentobarbital was injected to the radial vein with sterilized needles.

Table 2. Growth performance parameters of heat stressed broilers treated with vitamin C (1g/kg) for 6 weeks.

Parameters	Group 1	Group 2	Group 3	Group 4
Feed intake (g/bird)	1442 ± 10.3[a]	1328 ± 8.3[c]	1433 ± 8.6[a]	1382 ± 8.1[b]
B.W. gain (g/bird)	760 ± 9.9[a]	699 ± 7.6[c]	763 ± 5.0[a]	729 ± 9.0[b]
Feed conversion ratio	2.1 ± 0.01[a]	2.7 ± 0.01[c]	2.0 ± 0.1[a]	2.3 ± 0.01[b]
Mortality (%)	6[b]	18[a]	6[b]	10[b]
Dead birds	3[c]	9[a]	3[c]	5[b]

Group 1: Thermo-neutral birds (TN; 22°C for 24 hours/day) fed basal diet only.
Group 2: Heat stress birds (HS; 34°C for 8 hours/day) and fed on a diet only.
Group 3: Thermo-neutral birds (TN; 22°C for 24 hours/day) fed basal diet + Vitamin C.
Group 4: Heat stress birds (HS; 34°C for 8 hours/day) and fed basal diet + Vitamin C.

Determination of lipid peroxidation (MDA) and the activities of antioxidant enzymes (SOD, GST, GPX and CAT) in liver, heart and kidney tissues

The concentration of MDA (µM) was estimated by using Thiobarbituric acid reactive substance (TBARS) ELISA kits (10009055) from the Cayman Chemical Company, USA as described in the manufacturer's instructions. In addition, the ELISA kits provided by the same company were used for estimation of the activities of CAT (nmol/min/g tissue; catalogue #707002), GPX (nmol/min/g tissue; catalogue #703102), total SOD (U/g tissue; catalogue #706002) and GST (nmol/min/g tissue; catalogue #703302) on ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, USA). The results of enzymes activities calculated as described in the manufacturer's instruction.

RNA isolation and semi-quantitative real time RT-PCR analyses of heat shock proteins and heat shock factors gene expression in liver, heart and kidney tissues

Total RNA was extracted from homogenated pooled tissues using a TRIzol/choloroform/isopropanol method followed by the removal of supernatants. The RNA pellet was then dissolved in diethylpyrocarbonate (DEPC) treated RNase free water (Ambion, Texas, USA). DNA was removed using the a DNase I kit (Ambion, Austin, TX) and the RNA samples were checked for concentration and purity (260:280 nm absorbency). RNA (2 µg) was reverse transcribed to cDNA in a reaction mixture using an iScript cDNA synthesis Kit (Bio-Rad, Hercules, CA, USA). Relative-quantitative real-time RT-PCR was performed using a QuantiFast SYBR® Green PCR Kit (Qiagen, Valencia, CA, USA) on a Rotor-Gene Q Real-Time PCR system (Qiagen, Valencia, CA, USA). Briefly, the 20µl reaction mix was prepared from 10 µl of master mix; 2 µl forward primer (10 pmol); 2 µl reverse primer (10 pmol); 2 µl cDNA of the sample and 4 µl of nuclease-free water. The cycling parameters were 50°C for 2 minutes (first activation phase to SYBR® Green), 95°C for 15 minutes (second activation phase to SYBR® Green), 40 cycles at 95°C for 10 seconds (denaturation phase of cDNA), followed by 30 seconds at 55°C (annealing phase) and 72°C for 10 seconds (extension phase of cDNA) with final melting at 95°C for 20 seconds. The template concentration and the cycle number were optimized to ensure linearity of response and to avoid saturation of the reaction (40 cycles was better). Post-PCR melting curves confirmed the specificity of single-target amplification, and fold changes in gene expressions were normalized to housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). A duplicate from each cDNA was analysed, fluorescence emission was detected and relative quantification was calculated automatically. The primer sequences that were used in the real-time RT-PCR analyses were cGAPDH (F-5'GTGTTATCATCTCAGCTCCCTCAG'3; R-5'GGTCATAAGACCCTCCA CAATG3'), cHSP90 (F-5'ACTCTGCTTACCTTGTTGCGGA GA'3; R-5'TCCTTGTTCGCCGTTCTTCCAGA'3), cHSP70 (F-5'GGTAAGCACAAGCGTGACAATGCT'3; 5'TCAATCTCAATGCTGGCTTGCGTG'3), cHSP60 (F-5'ATGTGTGGAGCAGCAAGACAGAGA'3; R-5'TTCATGAGC TCCCAATCCCAGACA'3), cHSF-1 (F-5'TCCATGTGTTC GACCAAGGACAGT '3; R-5'TGGAACTCAGTGTCGTCCTTCTCT''3) and cHSF-3 (F-5AAATGCCACCAATGGTCTCCTTGC '3; R- CCCATTTATTGGGCTTGCTACA '3).

Statistical analysis

All statistical analyses were performed using IBM SPSS statistics 21 software for Mac OS (IBM software, Chicago, USA). Data were analyzed by using One-way analysis of variance (ANOVA). Differences were considered significant at $p<0.05$.

Table 3. The levels of malondialdehyde (MDA; µM), catalase (CAT; nmol/min/g tissue), glutathione peroxidase (GPX; nmol/min/g tissue), superoxide dismutase (SOD; U/g tissue) and glutathione-S transferase (GST; nmol/min/g tissue) of heat stressed broilers treated with vitamin C (1g/kg) for 6 weeks in liver, heart and kidney tissues.

Parameters	Tissues	Group 1	Group 2	Group 3	Group 4
MDA	Liver	15.1 ± 5.0^{b}	40.2± 1.6^{a}	17.2 ± 3.9^{b}	25.1 ± 4.3^{c}
	Heart	17.2 ± 2.1^{b}	45.2 ± 4.4^{a}	20.1 ± 4.3^{b}	30.0 ± 3.1^{c}
	Kidney	19.1 ± 3.4^{b}	43.2 ± 2.8^{a}	19.2 ± 3.2^{b}	32.6 ± 5.4^{c}
CAT	Liver	27.0 ± 2.2^{a}	15.5 ± 1.1^{b}	25.6 ± 2.0^{a}	20.1 ± 1.9^{c}
	Heart	28.4 ± 1.1^{a}	17.4 ± 1.2^{b}	27.1 ± 2.2^{a}	20.2 ± 1.2^{c}
	Kidney	29.2 ± 1.8^{a}	18.3 ± 1.1^{b}	26.1 ± 1.3^{a}	24.2 ± 1.1^{c}
GPX	Liver	522.3 ± 09.1^{a}	412.3 ± 10.0^{b}	532.1 ± 12.0^{a}	490.1 ± 10.4^{c}
	Heart	532.9 ± 10.6^{a}	420.5 ± 01.5^{b}	540.7 ± 08.0^{a}	500.3 ± 08.0^{c}
	Kidney	527.9 ± 07.3^{a}	416.7 ± 10.9^{b}	533.9 ± 08.5^{a}	486.4 ± 10.4^{c}
SOD	Liver	1.5 ± 0.1^{a}	0.5 ± 0.1^{b}	1.7 ± 0.4^{a}	1.0 ± 0.1^{c}
	Heart	1.6 ± 0.1^{a}	0.4 ± 0.1^{b}	1.8 ± 0.1^{a}	1.1 ± 0.1^{c}
	Kidney	1.4 ± 0.1^{a}	0.5 ± 0.1^{b}	1.5 ± 0.1^{a}	1.0 ± 0.1^{c}
GST	Liver	120.3 ± 5.1^{a}	92.2 ± 4.1^{b}	130.1 ± 9.9^{a}	100.9 ± 7.6^{c}
	Heart	130.8 ± 3.1^{a}	95.9 ± 2.6^{b}	120.2 ± 9.8^{a}	100.6 ± 2.1^{c}
	Kidney	125.8 ± 9.5^{a}	95.5 ± 5.2^{b}	120.8 ± 8.7^{a}	110.6 ± 6.1^{c}

$^{a\text{-}c}$ Within the same row, means ± SD with different superscripts differ significantly ($p<0.05$).
Group 1: Thermo-neutral birds (TN; 22°C for 24 hours/day) fed basal diet only.
Group 2: Heat stress birds (HS; 34°C for 8 hours/day) and fed on a diet only.
Group 3: Thermo-neutral birds (TN; 22°C for 24 hours/day) fed basal diet + Vitamin C.
Group 4: Heat stress birds (HS; 34°C for 8 hours/day) and fed basal diet + Vitamin C.

Results

Performance parameters

As indicated in Table 2, heat stress induced a significant ($p<0.05$) reduction in feed intake and body weight gain while the mortality percentage and feed conversion ratio were significantly increased in untreated heat stressed broilers chickens throughout the experiment. Interestingly, dietary inclusion of the examined dose of vitamin C (1g/kg basal diet) in the diet of heat stressed broilers alleviated the negative effect of heat stress as reflected in a significant increase in feed intake and body weight gain accompanied by a significant reduction in mortality percentage and feed conversion ratio throughout the experiment (Table 2).

Determination of lipid peroxidation (MDA) and activities of antioxidant enzymes (SOD, GST, GPX and CAT) in liver, heart and kidney tissues

The data summarized in Table 3 indicate that heat stress induced a significant elevation ($p<0.05$) of MDA in all examined tissues (liver, heart and kidney) compared to the control. Dietary supplementation of Vitamin C to thermo-neutral birds keeps the MDA concentration unchanged significantly ($p>0.05$) compared to control. However, addition of vitamin C to the basal diet of heat stressed broilers reduced the elevated MDA concentration significantly and reached values still higher than the control. The types of tissue examined were did not affect ($p>0.05$) the concentration of MDA. Heat stress induced a significant ($p< 0.05$) reduction of CAT (Table 3), GPX (Table 3), SOD (Table 3) and GST (Table 3) activities in all examined tissues compared to the control. Dietary supplementation of vitamin C to heat stressed chicks increased the activities of CAT, GPX, SOD and GST significantly ($p<0.05$) compare to that of untreated heat stressed birds, but lower than that of control. Regarding the types of tissue investigated, there were no significant differences ($p>0.05$) in the activities of the studied enzymes (Table 3).

RNA isolation and semi-quantitative real time RT-PCR analyses of heat shock protein and heat shock factor gene expression in liver, heart and kidney tissues

Real time PCR analysis of tested tissues indicated that, heat stress induced a significant ($p<0.05$) upregulation to HSP90 (Fig. 1A), HSP70 (Fig. 1B), HSP60 (Fig. 1C), HSF-1 (Fig. 1D) and HSF-3 (Fig. 1E) in all examined tissues (liver, heart and kidney) compared to the control. Dietary supplementation of Vita-

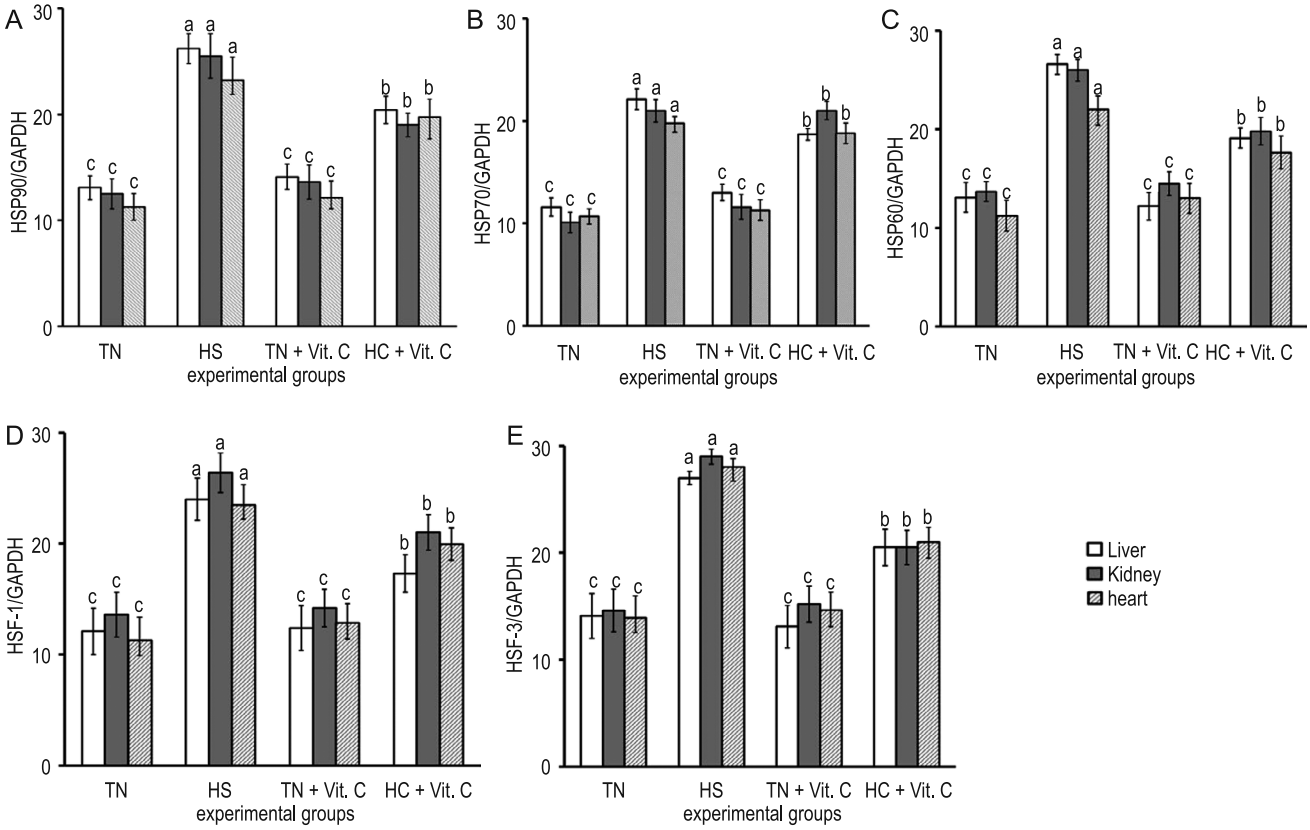

Fig. 1. mRNA levels of (A) HSP90, (B) HSP70 and (C) HSP60 (D) HSF-1 and (E) HSF-3 genes of thermoneutral (TN), heat stressed (HS) broiler chickens supplemented with or without vitamin C (1g/kg of basal diet) in liver, kidney and heart tissues. [a-c] Within group(s), means ± SD with different superscripts differ significantly ($p<0.05$).

min C to thermo-neutral birds maintains the expression of these genes at a level comparable ($p>0.05$) to that of controls. However, addition of vitamin C to the basal diet of heat stressed broilers downregulated the expression of these genes towards the control levels. Regarding the types of tissue investigated, there were no significant differences ($p>0.05$) in the levels of mRNA expression of the studied genes (Fig. 1).

Discussion

The observed reduction in feed intake and body weight gain and significant increase in the mortality percentage and feed conversion ratio in untreated heat stressed birds throughout the experiment agree with previous work which indicated the same detrimental effect of chronic heat stress in broilers (Al-Fataftah and Abu-Dieyeh 2007). Furthermore, the observed positive impact and ameliorative effect of vitamin C in relation to heat stress in broiler chickens as reflected in improved performance parameters and reduced mortality percentages agrees with earlier research (Khan et al. 2012). The significant higher level of MDA in untreated heat stressed broilers indicates the generation of a higher level of free radicals that overwhelms the antioxidant status of the birds (Whittow 1994). The observed significant higher level of MDA in the liver, kidney and heart tissues of untreated heat stressed broilers is consistent with earlier studies in broilers (Ismail et al. 2013, 2015), domestic chickens (Ramnath et al. 2008) and in heat stressed laying hens (Yardibi and Turkay 2008). The lowered levels of MDA in the examined tissues of heat stressed birds supplemented with vitamin C confirmed the ameliorative role of vitamin C against heat stress that was reported earlier in broiler chickens (Ismail et al. 2013, 2015). The significant reduction of CAT, GPX, SOD and GST activities in liver, kidney and heart tissues of untreated heat stressed birds as reported in the current study agrees with earlier works in broiler chickens subjected to heat stress in an open housing system (Ismail et al. 2015). Moreover, the significant enhancement of the activities of CAT, GPX, SOD and GST enzymes in liver, kidney and heart tissues of heat stressed birds treated with the current dose of vitamin C agrees with earlier reports in heat stressed broilers (Seven 2008, Ismail et al. 2013, 2015) and white leghorn layers (Panda et al. 2008) treated with ascorbic acid. All living organisms respond to environmental stressors, including hyperthermia, by synthesizing a set of proteins known as heat shock proteins (HSPs) (Becker and Craig 1994). HSPs are molecular chaperones essential for maintaining cellular functions by preventing misfolding and aggre-

gation of nascent polypeptides and by facilitating protein folding (Hartl and Hayer-Hartl 2002). Heat stress induced variations in the expression of HSP90, HSP70, HSP60, and these HSPs have a protective function in the cell (Lockwood et al. 2010). The significant increase in mRNA level of HSP90 in the studied tissues of untreated heat stressed birds of the current study disagree with previous work in broiler chickens (Yu et al. 2008, Zhao et al. 2013). This disparity might be due to differences existed in the experimental duration and stress criteria. Yu et al. (2008) exposed the birds to acute stress and not to chronic stress (42 days) as performed in the current study. Moreover, Zhao et al. (2013) subjected the birds to cold stress and not to heat stress as performed in the current study. The significant upregulation of mRNA expression in HSPs (70 and 60) in the studied tissues of untreated heat stressed birds as indicated in the current study are in accordance with previous work in broilers chickens (Yu et al. 2008, Yan et al. 2009, Zhao et al. 2013) and Pekin and Muscovy ducks (Zeng et al. 2014). In accordance with the current findings, a previous study (Chandrakant et al. 2012) indicated a significant reduction of HSP70 gene expression in heat stressed broilers treated with ascorbic acid (150mg/kg) in an extreme hot summer. On the other hand, the secretion of HSP70 in the liver and kidney was greater in heat stressed broilers treated with vitamin C compared to untreated heat stressed birds (Kapakin et al. 2012). This disparity may be attributed to different experimental methodology and protocols. In addition, the dose used in the earlier study (Kapakin et al. 2012) was a quarter of the current dose, which of course induced different action on the tested tissues. The significant upregulation of heat shock factors (HSF-1 and HSF-3) in untreated heat stressed birds of the current study explains the upregulation of HSP expression in these birds because HSP upregulation mechanism is dependent on upregulation of heat shock factors (HSF-1 and HSF-3) (Morimoto 1998, Pirkkala et al. 2001). The significant reduction of mRNA levels of HSPs in the liver, kidney and heart tissues of heat stressed birds treated with vitamin C was documented earlier for HSP70 in broiler chickens (Mahmoud et al. 2004) and heat stressed quails (Sahin et al. 2009). Many of the factors that induce the HSP response and the synthesis of the various types of heat shock proteins are also involved in the production of reactive oxygen species. Thus, oxidative stress has been proposed as a key mechanism that mediates HSP induction and biosynthesis (Mahmoud et al. 2004). Therefore, scavenging of reactive oxygen species and free radicals by the antioxidants (vitamin C) decreased the need for stimulation of HSPs gene expression (Mahmoud et al. 2004, Sahin et al. 2009). Based on the current findings, we can hypothesize that, heat stress causes increased oxidative stress with increased production of lipid peroxidation (MDA), increases the level of HSPs expression (Wang and Edens 1998, Mahmoud et al. 2003, Mahmoud and Edens 2005) and lowers the concentrations of antioxidant vitamins in liver, kidney and heart tissues (Sahin and Kucuk 2003). Furthermore, dietary supplementation of vitamin C to heat stressed birds alleviated the detrimental effects of heat stress as reflected on reduced lipid peroxidation (MDA) and activated the antioxidant enzymes with subsequent reduction of HSPs and HSFs levels. The current study can concluded that, dietary supplementation of the examined dose of vitamin C was efficient at ameliorating the detrimental effects of heat stress on liver, heart and kidney tissues of broilers chickens at cellular and molecular levels.

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