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Effects of Dietary Selenium, Vitamin E, and Their Combination on Growth, Serum Metabolites, and Antioxidant Defense System in Skeletal Muscle of Broilers Under Heat Stress

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Abstract This experiment was conducted to evaluate the effects of dietary vitamin E, selenium (Se), and a combination of the two, on the performance, serum metabolites and oxidative stability of skeletal muscle of broilers during heat stress. The broilers raised in either a thermoneutral (23.9°C constant) or heat stress (23.9°C to 37°C cycling) environment were assigned to 6 dietary treatments (0, 0.5, or 1 mg/kg Se; 125 and 250 mg/kg vitamin E; or 0.5 mg/kg Se plus 125 mg/kg vitamin E) from 1 to 49 days of age. At the end of the experiment, blood samples were collected from chicks, the chicks sacrificed, and pectoralis superficialis muscle was used for measurement of malondialdehyde (MDA) concentration and enzyme activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD). The heat-stressed chicks consumed less feed, gained less weight, and had higher feed conversion ratio when compared to thermoneutral chicks ($P< 0.05$). Serum concentrations of iron (Fe) and zinc (Zn) were decreased by heat stress ($P< 0.05$), whereas the serum concentrations of copper (Cu), glucose, and uric acid were significantly increased under heat stress ($P< 0.05$). The chicks that received supplemental of vitamin E exhibited significantly higher serum concentrations of Zn ($P< 0.05$) and significantly lower concentrations of Cu, glucose, and uric acid ($P< 0.05$) when exposed to heat stress. Dietary Se also caused a significant decrease in serum glucose, uric acid, and Cu concentrations of heat-stressed broilers ($P< 0.05$), but had no significant effect on Zn concentration ($P> 0.05$). The GPx activity remained relatively constant ($P> 0.05$), though SOD activity and MDA levels in skeletal muscle were enhanced on exposure to heat stress ($P< 0.05$). The heat-stressed chicks that received the combined supplementary level of vitamin E and Se had the lowest concentration of MDA and the highest activity of SOD in the skeletal muscle ($P< 0.05$). Dietary Se also caused a significant increase in enzyme activity of GPx in the skeletal muscle ($P< 0.05$). These results indicate that the derangement of blood parameters and oxidative stability in broilers under heat stress are improved by supplemental vitamin E and Se.

Keywords Heat stress · Selenium · Vitamin E · Serum metabolites · Oxidative stability · Broilers

Introduction

Heat stress is of major concerns for poultry production. Biochemical and physiological changes associated with hyperthermia can potentially promote reactive oxygen species (ROS) formation [9, 23]. Excessive levels of ROS result in the disturbance of balance between the oxidation and antioxidant defense systems, causing lipid peroxidation, oxidative damages to proteins and DNA [17] and biological molecules [1]. The impaired muscle membrane integrity in breast muscle of heat-stressed broiler chickens [31] was also considered to be related with the changed redox balance, because broiler chickens that were exposed to acute heat stress exhibited more than a twofold increase of MDA as an indicator for lipid peroxidation, in the skeletal muscle [22, 36].

Antioxidants play a major role in protecting cells from ROS by reducing free radicals and preventing the peroxidation of lipids [11, 26]. Previous studies demonstrate the

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environmental stress diminishes in vivo antioxidant status [29, 30]. Lower plasma concentrations of antioxidant vitamins such as vitamin C, E, and folic acid, and minerals like Zn and Se has been inversely correlated to increased oxidative damage in stressed poultry [5, 30].

Vitamin E is the major lipid soluble antioxidant present in the cell membrane and plays an important role as a chain-breaking lipid antioxidant and free radical scavenger in the membranes of cells and sub cellular organs [38]. Grau et al. [12] and Ryu et al. [27] reported that feeding poultry with higher level of dietary vitamin E increased the lipid oxidative stability of poultry meat. Other studies concluded that dietary supplementation of vitamin E was an effective approach for reducing oxidative deterioration in poultry [15].

Selenium is recognized as an essential trace element that plays an important role in antioxidant system as a component of Se-dependent glutathione peroxidase (GPx) [37, 39]. This enzyme, together with SOD and catalase, protects cells against damage caused by free radicals and lipoperoxides [8]. Selenium also enhances the actions of vitamin E in reducing peroxy radicals. In chickens, absorption of vitamin E is impaired by severe Se deficiency, and Se alleviates such deficiency by permitting higher levels of vitamin E to be absorbed [18]. In terms of nutritional values in poultry diets, reduction of body weight has been reported in chicks fed diets deficient in vitamin E and Se [20]. Swain et al. [35] suggests the deficiencies of vitamin E or Se, or both, can impair immune function in young chicks.

However, knowledge available on antioxidant status in the skeletal muscle tissue of heat-stressed broilers using vitamin E or Se, or both, is limited. In the present study, the effects of heat stress and of varying levels of supplemental vitamin E and Se, alone or in combination, on antioxidant status in the skeletal muscle tissue, and the metabolic responses of broilers reared under thermoneutral or heat stress conditions, were evaluated in broilers reared under thermoneutral and heat stress conditions.

Materials and Methods

Experimental Design and Chicks

The experimental protocol was approved by the guideline of Animal Ethics Committee of Razi University of Kermanshah (Iran). Two hundred and forty one-day-old broiler chicks (Cobb 500) were obtained from a local hatchery, weighed, and randomly assigned to 24 cages with 10 chicks per cage during the first 3 weeks of experiment. All cages were settled in two identical chambers that can be separated with a door in the same poultry house. The house had windows and was equipped with ventilation as gas heating systems controlled by thermostats. Each cage was allotted to one of four replicates for each of six dietary treatments from 1-day-old. The following six treatment diets were supplied to the chickens: No added Se and vitamin E (control), 0.5 mg of Se/kg of feed (SE1), 1 mg of Se/kg of feed (SE2), 125 mg of vitamin E/kg of feed (VE1), 250 mg of vitamin E/kg of feed (VE2), 0.5 mg of Se/kg of feed, and 125 mg vitamin E/kg of feed (SE1+VE1). Control was the basal diet (Table 1) containing 0.25 mg/kg Se and 33.20 mg/kg vitamin E. Vitamin E (α-tocopherol acetate) and Se (selenomethionine) were provided by a commercial company (Kiadane, Kermanshah). Water and feed were provided ad libitum; feed intake and body weights were recorded weekly.

Temperature Treatments

During the first 3 weeks of the experiment, chambers not separated and recommended brooding temperatures were applied. After 3 weeks at the recommended brooding temperatures, two of four cages per dietary treatment were subjected to either the high or the optimum temperature,

Table 1 Ingredients and nutrient level of basal diets

Ingredients (%)	Starter (0–21)	Grower (21–42)	Finisher (42–49)
Corn Seed	64.60	69.94	70.79
Soyabean meal, CP 48%	30.25	24.19	22.60
Soyabean oil	0.00	0.50	1.80
Fish meal	1.00	1.00	1.00
Dicalcium phosphate	1.60	1.57	1.44
Calcium carbonate	1.36	1.30	1.22
Salt	0.46	0.40	0.37
Mineral–vitamin premix[a]	0.50	0.50	0.50
DL-methionine	0.17	0.15	0.14
HCL-lysine	0.00	0.44	0.13
Nutrients composition			
Metabolizable energy (kcal/kg)	2,988	3,083	3,176
Crude protein (%)	21.05	19.00	18.00
Calcium (%)	1.01	0.96	0.90
Available phosphorus (%)	0.50	0.48	0.45
Methionine (%)	0.37	0.33	0.47
Methionine+cysteine (%)	0.85	0.76	0.74
Lysine (%)	1.16	1.31	1.03
Vitamin E analyzed (mg/kg)	33.20	33.60	36.00
Selenium analyzed (mg/kg)	0.25	0.24	0.24

[a] Mineral–vitamin premix provided the following per kilogram of diet: vitamin A, 5,500 IU; vitamin D3, 1,100 IU; vitamin E, 10 IU; riboflavin, 4.4 mg; vitamin B12, 12 mg; nicotinic acid, 44 mg; menadione, 1.1 mg; biotin, 0.11 mg; thiamine, 2.2 mg; and ethoxyquin, 125 mg, Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.17 mg; I, 0.46 mg; and Ca, minimum 150 mg and maximum 180 mg

and the hot temperature treatment chamber was separated from the thermoneutral chamber.

In one chamber, the ambient temperature was set to range between 23.9°C and 37°C to simulate the daily fluctuations of the diurnal temperatures during heat stress. The chicks were exposed for 8 h at 23.9°C, 4 h at 23.9 to 37°C, 8 h at 37°C, and for 4 h at 37 to 23.9°C. In the other chamber, temperature was kept at a constant 23.9°C. The relative humidity was allowed to fluctuate, but not to levels below 55%. During the next 4 weeks, the chicks were given the same dietary treatments as they received during the initial 3 weeks in the experiment, and the feed intake and body weights were monitored weekly. During the experiment, the chicks were maintained for 24 h on a constant lighting schedule, with an average light intensity of 15 lux.

Slaughter and Sampling Procedure

After the heat treatments, the chicks were weighed after an overnight feed deprivation and taken to the animal house slaughter facility. Blood samples were collected from chicks (four chicks per treatments per environmental chamber), the chicks were sacrificed, and portions of the pectoralis superficial muscle were rapidly excised. Tissues were immediately frozen in liquid nitrogen and powdered. Meanwhile sera were collected by centrifuging blood samples at 1,500×*g* for 20 min. Sera and tissues were stored at −20°C and −80°C, respectively.

Serum Parameter Measurements

Serum albumin, glucose, and uric acid were analyzed using the diagnostic kit (Pars Azmun, Iran) and enzymatic methods. The minerals, Fe, Zn, and Cu concentrations in serum were measured at specific wavelengths for each element (248.3, 213.9, and 324.8 for Fe, Zn, and Cu, respectively) by using an atomic absorption spectrometer (Younglin AAS-8,000) with Graphite Furnace Atomizer in deuterium background correction method. Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions of each element.

Determination of Skeletal Muscle MDA

Pectoralis superficialis muscle was used for MDA measurements after 1 week of storage at −80°C. Lipid peroxidation was assayed colorimetrically as a 2-thiobarbituric acid reactive substance (TBARS) using the modified method of Ohkawa et al. [25] described by Mujahid et al. [23]. The TBARS content was assayed by using a spectrophotometer (Hitachi U-2001, USA) at 532 nm and expressed as nmol of MDA per mg protein. Protein concentration was determined by the method of Bradford [4] using crystalline bovine serum albumin as a standard.

Measurement of Cu/Zn-SOD Activity

The activity of Cu/Zn-SOD in the pectoralis muscle of broiler chickens was measured using commercial kits from Cayman Chemical Company (Ann Arbor MI, USA). According to the manufacturer's instructions, Cu/Zn-SOD activity was assayed. Prior to measurement of SOD activity, tissue homogenates were diluted with sample buffer (diluted) 20 times to produce absorbances within the linear range of the standard curve. The absorbance of the sample and standard wells were monitored at 450 nm using a microplate reader (Bio-Radmodel 680, USA). SOD activity was expressed as U/mg protein. One unit of SOD is defined as the amount of enzyme needed to cause 50% dismutation of the superoxide radicals.

Determination of Glutathione Peroxidase Activity

GPx activity in the pectoralis muscle of broiler chickens was measured by using a kit available from Cayman Chemical Company (Ann Arbor MI, USA). According to the manufacturer's instructions, GPx activity was assessed at 340 nm by quantifying the rate of oxidation of NADPH to $NADP^+$. Prior to measurement of GPx activity, tissue homogenates were diluted with sample buffer (diluted) 25 times to produce absorbances within the linear range of the assay. The absorbance of the samples was monitored using a micro plate reader (Labsystems Multiskan MS-UV, Finland) at five time points spanning 5 min. GPx activity was expressed as U/mg protein.

Statistical Analysis

All the data were first analyzed using the general linear model procedure of SAS software [32] and differences among treatment means were determined using the least significance difference test. The two-way interactions of the study were entered in the second step, and analyses of simple main effects were performed using the Lsmeans/Slice feature in the Proc Mixed statement. Interaction between dietary treatments and environmental temperature was sliced by temperature to compare dietary treatments separately at each environmental temperature.

Results

Growth Performance

The effect of different levels of vitamin E and Se on growth performance of 49-day-old broilers under heat stress is shown in Table 2. No differences among dietary treatments were observed in performance characteristics ($P>0.05$). There was a significant reduction in body weight, feed

Table 2 Body weight, feed conversion, and intake of 49-day-old broilers fed different levels of vitamin E and selenium under thermoneutral (TN) and heat stress (HS) conditions

Diets	Body weight (g)		Feed Intake (g)		Feed Conversion (g/g)	
	TN	HS	TN	HS	TN	HS
C	2,136.14	1792.50	4282.53	3930.43	2.01	2.19
SE1	2,263.72	1764.29	4584.77	4011.69	2.02	2.27
SE2	2,082.19	1797.77	4270.82	3932.28	2.05	2.19
VE1	2,236.32	1648.83	4347.41	3620.29	1.94	2.19
VE2	2,156.72	1767.37	4283.26	3697.35	1.99	2.10
SE1+VE1	2,238.47	1870.23	4276.43	3798.33	1.91	2.04
SEM	51.65		66.33		0.02	
Source of variation (*P* values)						
Diet	0.91		0.31		0.28	
Temperature	0.0001		0.0001		0.001	
Diet×Temperature	0.82		0.74		0.88	
Main effect means						
Diet						
C	1,964.32		4,016.48		2.10	
SE1	2,014.01		4,298.23		2.15	
SE2	1,939.99		4,096.55		2.12	
VE1	1,942.58		3,983.95		2.07	
VE2	1,962.05		3,990.31		2.04	
SE1+VE1	2,054.35		4,037.38		1.97	
Temperature						
TN	2,185.60[a]		4,340.87[a]		1.98[b]	
HS	1,773.50[b]		3,830.06[b]		2.16[a]	

Means within a column showing different superscripts are significantly different ($P<0.05$); least significance difference test was applied to compare means. Data were from 20 chicks per treatment

C control, *SE1* 0.5 mg/kg selenium, *SE2* 1 mg/kg selenium, *VE1* 125 mg/kg vitamin E, *VE2* 250 mg/kg vitamin E, *SE1+VE1* 0.5 mg/kg selenium+125 mg/kg vitamin E, *SEM* standard error of the mean

intake, and conversion ratio when the chicks were exposed to heat stress ($P<0.05$). There was not a significant interaction in broiler growth performance between dietary treatments and environmental temperature ($P>0.05$).

Serum Metabolic Variables

Albumin, Glucose, and Uric Acid Concentrations

Table 3 shows the effect of different levels of vitamin E and Se on serum albumin, glucose, and uric acid concentrations of heat-stressed broilers. The serum albumin concentrations were not affected in treated chicks and controls ($P>0.05$). However, relative to control and non-treated chicks, the serum glucose and uric acid were significantly increased ($P<0.05$) in heat stressed broilers. None of the above mentioned parameters were significantly influenced by differences in dietary vitamin E or Se under thermo neutral condition ($P>0.05$), whereas both glucose and uric acid concentrations were affected by vitamin E and Se in heat stress condition ($P<0.05$). However, uric acid concentration of broilers fed 125 mg/kg of vitamin E was significantly lower than that of the other diets ($P<0.05$), whereas no significant difference was observed in uric acid concentration of broilers supplemented with 250 mg/kg of vitamin E compared with the control treatment ($P>0.05$).

Fe, Zn, and Cu Concentrations

The observed Fe, Zn, and Cu concentrations are given in Table 4. It may be seen that the heat-stressed chicks had lower ($P<0.05$) serum concentrations of Zn and Fe, but higher ($P<0.05$) concentrations of Cu compared to the chicks in the control or non-treated groups. Neither vitamin E nor Se caused changes of the serum concentrations of Fe, Zn, and Cu under thermo neutral conditions ($P>0.05$), whereas dietary vitamin E resulted an increase ($P<0.05$) of the serum concentrations of Zn, but a decrease ($P<0.05$) of the serum Cu concentrations in the chicks exposed to heat stress. Dietary supplemental Se at 1 mg/kg Se also caused a significant reduction of the serum Cu concentrations of heat-stressed broilers ($P<0.05$).

MDA Concentration and Enzymatic Scavenger Activity

Table 5 shows the skeletal muscle MDA concentrations and antioxidant enzyme activities in thermoneutral and heat stress conditions. Compared to control chickens, exposure

Table 3 Serum albumin, glucose, and uric acid concentrations of 49-day-old broilers fed different levels of vitamin E and selenium under thermoneutral (TN) and heat stress (HS) conditions

Diets	Albumin (mmol/L)		Glucose (mmol/L)		Uric acid (mmol/L)	
	TN	HS	TN	HS	TN	HS
C	145.33	151.86	22.53	33.31[a]	59.48	76.39[a]
SE1	150.85	155.96	23.60	27.66[b]	55.88	71.87[ab]
SE2	149.70	143.46	23.02	27.53[b]	53.30	69.41[b]
VE1	156.22	150.46	24.45	27.04[b]	56.56	61.44[c]
VE2	154.94	149.26	21.71	28.83[b]	55.93	75.78[a]
SE1+VE1	153.66	148.06	23.61	26.68[b]	54.32	62.66[c]
SEM	1.40		0.68		1.73	
Source of variation (*P* values)						
Diet	0.77		0.07		0.002	
Temperature	0.55		0.0001		0.0001	
Diet × Temperature	0.70		0.005		0.01	
Main effect means						
Diet						
C	148.59		27.92		67.93	
SE1	153.40		25.63		63.87	
SE2	146.58		25.27		61.35	
VE1	153.34		25.74		59.00	
VE2	152.10		25.27		65.85	
SE1+VE1	150.86		25.14		58.49	
Temperature						
TN	151.78		23.15		151.78	
HS	149.84		28.51		149.84	

Means within a column showing different superscripts are significantly different ($P < 0.05$); least significance difference test was applied to compare means. Data were from four chicks per treatment

C control, *SE1* 0.5 mg/kg selenium, *SE2* 1 mg/kg selenium, *VE1* 125 mg/kg vitamin E, *VE2* 250 mg/kg vitamin E, *SE1+VE1* 0.5 mg/kg selenium+125 mg/kg vitamin E, *SEM* standard error of the mean

to heat treatment significantly increased the skeletal muscle MDA concentration ($P < 0.05$). Heat-treated broilers showed an elevation in skeletal muscle Cu/Zn-SOD activity ($P < 0.05$), whereas no significant changes were detected in GPx activity in the skeletal muscle of chickens on exposure to heat stress ($P > 0.05$). Dietary vitamin E had a significant effect on MDA concentration and Cu/Zn-SOD in the skeletal muscle, but the enzyme activity of GPx remained depressed in response to dietary vitamin E ($P < 0.05$). Dietary supplemental of Se on the other hand causes an increase ($P < 0.05$) in enzyme activity of GPx and subsequently a reduction ($P < 0.05$) in MDA concentration in heat-stressed broilers ($P < 0.05$). Selenium alone had no effect on the Cu/Zn-SOD activity; however, Se together with vitamin E supplementation had synergistic effect.

Discussion

An ambient temperature above 32°C is considered to have an adverse effect on the performance of broiler chicks. Earlier findings have suggested that reduced feed intake, body weight, and feed conversion efficiency are caused by high environmental temperatures [2, 22]. However, supplementing the diet with vitamin E and Se can alleviate some of these adverse effects on growth performance, attributed to high ambient temperatures [28, 30]. In this study, there was a significant reduction in growth performance when the chicks were exposed to heat stress. However, no differences among dietary treatments were observed in performance characteristics either in thermoneutral or heat stress conditions. The lowest concentrations of vitamin E and Se employed in this study were 33.2 and 0.24 mg/kg diet, which are, respectively, about three and two times higher than the National Research Council [24] recommendation for broiler chicks. These suggest that the basal levels of vitamin E (33.20–36.00 mg/kg diet) and Se (0.25–0.24 mg/kg diet) were adequate to maintain the optimum growth under normal condition or to prevent further reduction of growth under heat stress condition.

The maintenance of redox balance depends on the production and quenching of ROS. To resist damage caused by the presence of ROS, organisms have evolved nonenzymatic and enzymatic antioxidant defense mechanisms. The nonenzymatic antioxidant defense mechanisms include direct free radical scavengers, such as vitamin E, vitamin C [6], albumin [13], uric acid [34], and several iron chelators, such as ferritin [14]. The enzymatic defenses are provided by SOD, GPx, as well as catalase and glutathione reductase with theirs coordinating mineral, Zn, Cu, Mg, and Se [3],

Table 4 Serum Fe, Zn, and Cu concentration of 49-day-old broilers fed different levels of vitamin E and selenium under thermoneutral (TN) and heat stress (HS) conditions

Diets	Fe (μg/dL)		Zn (μg/dL)		Cu (μg/dL)	
	TN	HS	TN	HS	TN	HS
C	405.10	280.00	150.00	100.50^{b}	16.50	25.20^{a}
SE1	402.00	282.00	153.00	104.30^{b}	16.24	24.40^{ab}
SE2	450.10	292.00	153.10	107.40^{b}	16.20	22.10^{bc}
VE1	408.50	312.50	152.60	122.30^{a}	15.71	21.10^{c}
VE2	412.40	325.00	155.10	132.70^{a}	15.00	16.49^{d}
SE1+VE1	407.00	338.30	155.60	125.10^{a}	14.95	19.79^{c}
SEM	11.92		4.39		0.76	
Source of variation (*P* values)						
Diet	0.56		0.001		0.001	
Temperature	0.0001		0.0001		0.0001	
Diet × Temperature	0.74		0.01		0.03	
Main effect means						
Diet						
C	342.55		125.25		20.85	
SE1	342.00		128.65		20.32	
SE2	348.55		130.25		19.15	
VE1	360.50		137.45		18.41	
VE2	368.70		143.40		15.74	
SE1+VE1	372.65		140.35		17.37	
Temperature						
TN	406.69^{a}		153.23		15.76	
HS	304.97^{b}		115.21		21.15	

Means within a column showing different superscripts are significantly different ($P < 0.05$); least significance difference test was applied to compare means. Data were from four chicks per treatment

C control, *SE1* 0.5 mg/kg selenium, *SE2* 1 mg/kg selenium, *VE1* 125 mg/kg vitamin E, *VE2* 250 mg/kg vitamin E, *SE1+VE1* 0.5 mg/kg selenium+125 mg/kg vitamin E, *SEM* standard error of the mean

which detoxify peroxides and protect the cells from subsequent deleterious effects.

In our present study, the glucose and uric acid concentrations in serum were increased when chicks exposed to heat stress, whereas supplementing dietary vitamin E at 125 mg/kg or Se at 1 mg/kg, independently alleviated the negative effects of high environmental temperature on serum glucose and uric acid. Neither levels of vitamin E and Se nor heat stress significantly altered serum albumin concentration. Although we did not measure corticosterone levels, it seems the higher serum concentrations of uric acid and glucose are associated with increased concentrations of corticosterone following exposure to heat stress [19, 40]. Uric acid concentration has been shown to increase in corticosterone-treated poultry as a result of corticosterone-induced muscle catabolism [16, 33]. Elevation in corticosterone also may have elicited gluconeogenesis, in which amino acids are converted to glucose, and therefore blood glucose levels increased [21, 33]. Marked decreases in serum concentrations of glucose and uric acid with supplemental dietary Se and vitamin E were probably due to the reduction of catabolic effect (or concentration) of corticosterone [7, 10]. In addition, we observed the increases in enzymatic antioxidants activities in Se and vitamin E supplemented broilers under heat stress condition, suggesting Se and vitamin E could improve antioxidant capacity in body and meanwhile protect the muscle cell membrane to inhibit protein catabolism, bringing about decrease in uric acid and glucose.

Heat stress in this experiment led to increased concentration of Cu but decreased Fe and Zn concentrations. Although the serum concentration of Fe remained unchanged, the chicks that received supplemental vitamin E under heat stress had significantly higher serum Zn level and significantly lower serum Cu than that of received control diet. Dietary supplemental of 1 mg/kg Se also caused a significant reduction in serum Cu concentration of heat-stressed broilers compared to untreated or those received 0.5 mg/kg Se. These results were not surprising as similar results are well documented by Sahin et al. [29, 30]. However they reported also that increasing both dietary Se and vitamin E caused an increase in serum concentrations of Fe. It was likely due to the low number of blood samples, the different levels of dietary Se and vitamin E, as well as less severe extent of stress compared with aforementioned studies.

In accordance with previous works [2, 22], heat stress could induce lipid peroxidation at 4 weeks after treatment, as we found the MDA concentration in skeletal muscle was enhanced about 2.7 times in untreated chicks exposed to

Table 5 Malondialdehyde (MDA) concentration and enzyme activities of SOD and GPx in skeletal muscle (per mg wet tissue protein) of 49-day-old broilers fed different levels of vitamin E and selenium under thermoneutral (TN) and heat stress (HS) conditions

Diets	MDA (nmol/mg protein)		SOD (U/mg protein)		GPx (U/mg protein)	
	TN	HS	TN	HS	TN	HS
C	0.81	2.16[a]	300.53	472.60[c]	3.37	3.52[bc]
SE1	0.71	1.46[b]	290.50	538.34[c]	3.27	3.90[ab]
SE2	0.78	1.38[b]	290.69	540.21[c]	3.33	3.84[ab]
VE1	0.79	1.46[b]	294.24	549.28[b]	3.48	3.62[abc]
VE2	0.78	1.43[b]	315.68	524.50[b]	3.50	3.27[c]
SE1+VE1	0.83	1.17[c]	303.03	607.99[a]	3.53	4.16[a]
SEM	0.08		25.95		0.07	
Source of variation (*P* values)						
Diet	0.0001		0.04		0.03	
Temperature	0.0001		0.0001		0.001	
Diet × Temperature	0.0001		0.03		0.03	
Main effect means						
Diet						
C	1.48		386.57		3.44	
SE1	1.12		389.42		3.58	
SE2	1.10		390.46		3.58	
VE1	1.08		421.77		3.55	
VE2	1.08		420.10		3.39	
SE1+VE1	1.00		455.51		3.85	
Temperature						
TN	0.78		299.11		3.41	
HS	1.51		538.82		3.72	

Means within a column showing different superscripts are significantly different ($P < 0.05$); least significance difference test was applied to compare means. Data were from four chicks per treatment

C control, *SE1* 0.5 mg/kg selenium, *SE2* 1 mg/kg selenium, *VE1* 125 mg/kg vitamin E, *VE2* 250 mg/kg vitamin E, *SE1+VE1* 0.5 mg/kg selenium+125 mg/kg vitamin E, *SEM* standard error of the mean.

heat stress. Accompanied by this increase in MDA concentration, untreated chicks subjected to the heat stress exhibited an increase in skeletal muscle Cu/Zn-SOD activity, while the GPx activity remained relatively constant in these chicks. This kind of response in untreated chicks could be unfavorable to their body system, suggesting that chicks exposed to heat stress may have entered an initial stage of changes to the antioxidant enzyme system in which the Cu/Zn-SOD activity increased in the cytosol to protect against surplus O_2^-, but then failed to activate GPx in skeletal muscle. This may be one the reasons that induce lipid peroxidation in the cytosol of the skeletal muscle of heat-stressed chickens, even though Cu/Zn-SOD activity increases. The present study for the MDA values, showed a decrease in all dietary treatments. In addition, the combined supplementary level of 125 mg/kg vitamin E and 0.5 mg/kg Se were the most effective inhibitor of lipid oxidation. The results also showed that dietary vitamin E cause a significant increase in Cu/Zn-SOD in the skeletal muscle of heat-stressed broilers. Selenium together with vitamin E supplementation had synergistic effect on the Cu/Zn-SOD activity; however Se alone had no effect. The enzyme activity of GPx however remained depressed in response to dietary vitamin E, Se supplementation but caused an increase in enzyme activity of GPx at both levels. These results on one hand confirmed previous results [3, 30] that Se and vitamin E exert their antioxidative activity in biological system via different manners. Vitamin E is present in the membrane components of the cell and prevents peroxide formation [38], whereas Se functions throughout the cytoplasm as a component of selenoenzyme GPx to destroy peroxides [37, 39]. Besides, the results clearly showed that dietary Se and vitamin E when used together had synergistic effect on the Cu/Zn-SOD activity in skeletal muscle. This may be another reason for the synergistic effect of vitamin E and Se on MDA concentration in heat-stressed broilers. Although the exact mechanism of this association is still not fully understood, our data suggest that vitamin E and Se may influence Zn and (or) Cu metabolism, thus indirectly affecting cytosolic SOD activity in heat-stressed chicks.

Conclusions

Although the levels of Se and vitamin E used in this study did not impact the growth performance of the chicks, supplementing dietary vitamin E or Se independently caused the beneficial effects on some blood parameters of heat-stressed broilers. Accompanied by reduction of MDA values

in the skeletal muscle, inclusion of combined supplementary of Se and vitamin E in diets could substantially increase the enzyme activity of Cu/Zn-SOD on exposure to heat stress, whereas dietary Se alone had no effect in improving Cu/Zn-SOD. The enzyme activity of GPx on the other hand remained depressed in response to dietary vitamin E and Se at both levels but caused an increase in enzyme activity of GPx in heat-stressed broilers. Taken together the present study indicated that a combination of dietary vitamin E (125 mg/kg) and Se (0.5 mg/kg) offers a good management practice to reduce heat stress-related disturbances in oxidative stability of skeletal muscle of broiler chicks.

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