The effects of dietary oleuropein and organic selenium supplementation on performance and heat shock protein 70 response of brain in heat-stressed quail

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Abstract

The study aimed to investigate whether dietary supplementation of oleuropein (O) alone or with organic selenium (OSe) will show the similar effects as the dietary supplementation of α-tocopherol acetate (TA) on performance, blood Heterophil/Lymphocyte ratio (H/L ratio) and brain Hsp70 gene in Japanese quails reared under heat stress (34°C). A total of 800 Japanese quails were kept in wire cages in a temperature-controlled room at either 22°C (thermonutral, TN) or 34°C (heat stress, HS) for 8 h/days and fed 5 different dietary treatments. Quails were fed on a basal diet (NC) or the diets supplemented with TA (TA200) or O (O200) at 200 mg/kg alone or with OSe (TA200+OSe and O200+OSe) to NC. HS decreased the final body weights (BW) (P<0.05), the body weight gains (BWGs) (P<0.01) and feed intake (FI) (P<0.05) and also deteriorated feed conversion ratio (FCR) (P<0.05) of quails from 21 to 35 d of age. Moreover, feeding with the TA200, O200, TA200+OSe and O200+OSe diets increased the final BWs and BWGs and FI of quails from 21 to 35 d of age (P<0.05). While HS increased blood H/L ratio (P<0.05) and brain Hsp70 gene expression (P<0.001). All dietary treatments except the NC diet on blood H/L ratio and O200+OSe on brain Hsp70 gene expression had positive effects. Consequently, the present study showed that supplementation of O alone or with OSe eliminated the deleterious effects of heat stress on growth performance, blood H/L ratio and brain Hsp70 gene expression of Japanese quails.

Introduction

Heat stress (HS) is one of the most challenging environmental conditions affecting poultry production in many regions of the world, which causes adverse effects including decreased growth performance, high mortality and large economic losses in poultry (Gu et al., 2012; Kamboh et al., 2013; Sahin et al., 2013). The internal homeostasis of poultry is constantly challenged by intrinsic and extrinsic stressors (e.g., heat stress, immune challenges). As a result of this, HS impaired the redox homeostasis that is maintained by pro-oxidant/antioxidant balance. The imbalance in favour of the pro-oxidant system resulted in increased levels of reactive oxygen species (ROS) in mitochondria and oxidative stress, which is causing lipid peroxidation and oxidative damages to proteins and DNA (Lin et al., 2006; Pamok et al., 2009). Such oxidation increased levels of ROS that can attack and irreparably damage membrane composition and permeability and impaired antioxidant status in poultry in vivo (Mahmoud et al., 2004; Sahin et al., 2013). As a consequence, the body starts producing and releasing heat shock proteins (Hsps) to try and protect itself from the deleterious cellular effects of ROS. The Hsps, called stress proteins, have an important role in the protection and repair of cells and tissues against high or low temperature or other stressors (Gu et al., 2012). Among these protein families, Hsp70 plays roles in protein folding and cell translocation and is highly stress-inducible as a biomarker of stress (Dridi et al., 2013). In fact, higher concentrations of Hsp70 were found in broilers and laying hens exposed to HS (Droge, 2002). Moreover, the corticotropin releasing hormone (CRH) that is secreted by the paraventricular nucleus of the hypothalamus in response to HS initiated the secretion of the adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary gland in poultry. The raising of blood corticosterone levels in the poultry under HS caused the rapid influx of heterophils into blood from bone marrow that increased the concentration of circulating heterophils. As a result, HS causes an increase in the ratio of Heterophil to lymphocyte (H/L) due to the reduced numbers of circulating lymphocytes and the higher numbers of heterophils in plasma of poultry (Lara and Rostagno, 2013; Sahin et al., 2013). Moreover, it is reported that the H/L ratio in blood is a more enduring and reliable indicator for the long-term stresses including HS than assessing corticosterone levels in poultry (Altan et al., 2000; Zulkifi et al., 2009; Kamboh et al., 2013). In addition, HS decreased the absorption and the concentrations of vitamins A, E and C and minerals such as Se, Zn, Mg etc. in the plasma and tissue. Heat stress also reduced the activities of the cellular antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase. As a result, this stress increases the requirement of these vitamins and minerals (Sahin et al., 2002; 2009a). In this respect, several studies that the antioxidants such as vitamin E and C and Se, Zn, Mg etc. alone (Sahin et al., 2009a; Abidin and Khatoon, 2013) or in combination (Sahin and Kucuk, 2001; Sahin et al., 2006) were supplemented to diets, are available for amelioration the negative effects of HS in poultry. Vitamin E and Se are key components of the antioxidant system, reducing lipid peroxidation.

Vitamin E is known to be a major chain-breaking antioxidant that protects cells and tissues from liperoxidative damage induced by free radicals (Jena et al., 2013). Selenium (Se) plays an important role in the antioxidant defence system due to its function in the active site of Se dependent glutathione peroxidase (GSHPx), which is involved in cellular antioxidant protection. It has been suggested that there is a synergistic relationship between Se and Vitamin E, because GSHPx continues the work of vitamin E by detoxifying hydroperoxides (Suriar, 2002). Inorganic (selenium, seleniumate, selenide, selenite) and organic (Se enriched yeast or algae) forms of Se may be used as supplement in the animal diets. On the other hand, in recent years, the organic Se sources have been widely used as an alternative to the inorganic Se because organic Se is deposited into the...
animal tissue more efficiently than inorganic Se (Özkan et al., 2007; Zdunczyk et al., 2013). Apart from vitamin C and E, flavonoids- and polyphenols-rich foods, such as fruits, vegetables and beverages including fruit juices, wine, tea, olive-leaf and oil have been receiving much attention as natural antioxidants (Hayes et al., 2011). In the last years, limited attention has been directed to other potential sources of antioxidant phytochemicals such as the leaves of the olive trees (Olea europaea L.). Oleuropein being the most prominent and active phenolic compound may reach concentrations of 60-90 mg/g dried olive leaves (Benavente-Garcia et al., 2000). Most of these phenolic compounds found in the olive leaf or olive leaf extract have been shown in vitro (Chimi et al., 1991; Visioli and Gali, 1994; Silva et al., 2006) and in vivo (Endgecombe et al., 2000; Ruiz-Gutierrez et al., 1995, 2001; Andreadou et al., 2006; Jemai et al., 2008) antioxidant activity. Le Tutour and Guedon (1992) and Aruoma et al. (1998) reported that olea europaea extracts, oleuropein and hydroxytyrosol were much more effective than BHT or vitamin E with regard to their antioxidative activities.

The ability of the bioactive polyphenolic compounds in the olive leaves extract to act as a free radical scavenger is partly related to their standard one-electron reduction potential by either donating hydrogen or electron or breaking the free radical chain reaction (Hayes et al., 2011; Lee and Lee, 2010). In addition, these bioactive compounds may also contribute to their antioxidant activity by preventing metal ion chelation (Endgecombe et al., 2000; Visioli et al., 2002; Hayes et al., 2011).

The objective of the present study was to search out whether or not the dietary supplementation of oleuropein (O) alone or with organic selenium (OSe) will show the similar effects with those of the dietary supplementation of α-tocopherol acetate (TA) alone or with OSe in terms of performance, blood Heterophil/Lymphocyte ratio (H/L ratio) and brain Hsp70 gene in Japanese quails reared under heat stress (34°C).

Materials and methods

Animal care and housing

A total of 800 one-day old mixed sex Japanese quail (Coturnix coturnix japonica) chicks purchased from a commercial hatchery (University Poultry Production and Marketing Plant, Samsun, Turkey) were used in the experiment. The study was conducted in accordance with animal welfare at the Poultry Research Centre of Gaziosmanpasa University. The chicks were weighed and randomly distributed to the 40 wire cages (20 chicks/cage). Each cage was equipped with nipple drinkers and electrical heating system by thermostats. The poultry research house including of these cages had two identical rooms that can be separated with a door. During the first 14 days of the experiment, rooms were not separated and standard brooding temperatures were applied to both rooms with temperature gradually decreased from 32°C to 26°C by the end of the second week of age. Quails in each wire cages were randomly assigned to 10 experimental groups, 4 replicates of 20 quails each in a 2 (temperature treatments) x 5 (dietary treatments) factorial arrangement from 14 to 35 days of age. At the 14th days, the experiment rooms were separated from each other and 5 of 10 experimental groups were subjected to either thermoneutral temperature or heat stress treatments. Applied temperatures in the rooms were as follows:

In thermoneutral temperature room, quails were kept at 24°C, 22°C and 20°C, at 21, 28 and 35 days. Relative humidity in this room ranged from 50% to 60% during the experiment.

In heat stress temperature room, quails were exposed to 34°C for 8 h/d (from 09:00 to 17:00) and then (from 17:00 to 09:00 h) to 24°C, 22°C and 20°C at 21, 28 and 35 days. Relative humidity ranged from 60% to 70% from the 14th day until the end of the study. The experiment was conducted during Autumn.

Temperature and humidity were monitored in each room at two locations using a temperature-humidity recording system. A fluorescent lighting schedule of 23 h light and 1 h dark was used during the study with an average light intensity of 40 lux/m².

Diets

Quails in both temperatures were fed one of 5 different diets in mash form until 35 days of age. The 5 experimental diets were as follows: the basal diet included 0.15 mg/kg sodium selenite (inorganic Se) and 50 mg α-tocopherol acetate (vitamin E)/kg diet and diets were formulated by supplementing of 2 different antioxidant sources (α-tocopherol acetate (vitamin E) or oleuropein (olive leaf extract) at 200 mg/kg level alone or with organic Se (Sel-Plex) at 0.30 mg/kg level to the basal diet. Feed ingredients were ground through a 1 mm screen in preparation for chemical analysis. Prior to experimental diet formulation, feed ingredients were analysed for crude protein (CP), ether extract, starch and total sugar according to the methods of the AOAC (2007).

Metabolisable energy (ME) of feed ingredients was calculated based on analysed values of feedstuffs (WPSA, 1989). All diets were formulated to meet minimum nutrient requirements established by the NRC (1994). The ingredients and calculated nutritional composition of the basal diet were given in Table 1. The α-tocopherol acetate (TA) was supplied from Kartal Chemistry Ltd. (Izmit, Turkey). Organic Se was provided as Sel-Plex® 2000 from Alltech Biotechnology Food, Agricultural and Animal Products Company (Bornova, Izmir, Turkey). Vitamin premix and trace mineral premix were provided from Topkim-Topkapı Drug Company Ltd. (Istanbul, Turkey).

The olive leaf extract was provided by the Bio-Olive Ltd. Company (Akyalik, Turkey). Provided olive leaf extract was filtered with Whatman No.1 filter paper and then the filtrates were carried to a rotary evaporator to evaporate the water.

Table 1. The ingredients and nutritional composition of the basal diet fed to quails from hatch to 5 weeks of age (as fed basis).

| Ingredients, g/kg |                  |
|------------------|------------------|
| Maize            | 400.7            |
| Wheat            | 100.9            |
| Soybean meal     | 345.0            |
| Full-fat soybean | 113.8            |
| Soybean oil      | 12.0             |
| Dicalcium phosphate | 8.5             |
| Limestone        | 12.2             |
| DL-methionine    | 0.2              |
| Sodium chloride  | 3.2              |
| Vitamin premix†  | 2.5              |
| Trace mineral premix* | 1.0            |

Chemical composition (calculated)

| Metabolisable energy, MJ/kg | 12.15 |
| Dry matter, g/kg           | 884.0 |
| Crude protein, g/kg        | 240.0 |
| Ca, g/kg                   | 8.0   |
| P (available), g/kg        | 3.0   |
| Methionine, g/kg           | 5.0   |
| Methionine+Cystine, g/kg   | 7.6   |
| Lysine, g/kg               | 13.9  |
| Threonine, g/kg            | 9.0   |
| Tryptophan, g/kg           | 3.5   |

Chemical composition (analyzed)

| Dry matter, g/kg | 885.2 |
| Crude protein, g/kg | 239.4 |
| Crude fat, g/kg    | 50.2  |
| Crude fibre, g/kg  | 40.4  |

*Vitamin premix provided per kg of diet: retinyl acetate, 4.128; cholecalciferol, 0.015; α-tocopherol acetate, 50 mg; menaquinone-4, 5 mg; thiamine, 3 mg; riboflavin, 6 mg; pyridoxine, 5 mg; cyanocobalamin, 0.03 mg; niacin, 25 mg; calcium-D-pantothenate, 12 mg; folic acid, 1 mg; D-biotin, 0.05 mg; apo-carotenoid acid ester, 2.5 mg; choline chloride, 400 mg. †Trace mineral premix provided per kg of diet: Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.20 mg; I, 1 mg; Se, 0.15 mg.

†Vitamin premix provided per kg of diet: retinyl acetate, 4.128; cholecalciferol, 0.015; α-tocopherol acetate, 50 mg; menaquinone-4, 5 mg; thiamine, 3 mg; riboflavin, 6 mg; pyridoxine, 5 mg; cyanocobalamin, 0.03 mg; niacin, 25 mg; calcium-D-pantothenate, 12 mg; folic acid, 1 mg; D-biotin, 0.05 mg; apo-carotenoid acid ester, 2.5 mg; choline chloride, 400 mg. †Trace mineral premix provided per kg of diet: Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.20 mg; I, 1 mg; Se, 0.15 mg.
remove the ethanol under reduced pressure at 38°C, 120 rpm. The remaining aqueous solutions were lyophilized at -50°C, 0.028 mbar and the crude extracts were kept in vacuum bags at -80°C until the use.

**Analytical procedures**

Total phenol contents of the olive leaf extract (OLE) and the experimental diets were determined by using Folin-Ciocalteu method with a modification of Lako et al. (2007). According to this method, crude extract was dissolved in dimethyl sulfoxide (DMSO) in a ratio of 1 g extract in 20 mL DMSO. Five hundred μL OLE or standard (gallic acid) solutions were mixed with 2.5 mL Folin-ciocalteu reagent (1:10 dilution with deionized water) and left to stand 2.5 min at room temperature and then 2 mL of sodium carbonate solution (7.5% in deionized water) was added. After incubating 1 h at room temperature in a dark environment, the absorbances were measured at 725 nm by UV spectrophotometer (Perkin Elmer, Germany). Results were expressed as milligrams of gallic acid equivalents (GAE) per fresh weight. The phenol content of OLE was calculated as 196.81±2.83 mg/GAEq/g extract. The oleuropein (O) content of the OLE and experimental diets were analysed in the Sciences of Izmir Institute of Technology (Izmir, Turkey). The high performance liquid chromatography (HPLC) analysis was used for the determination of the oleuropein content in the OLE and diets supplemented with oleuropein at different levels. The HPLC equipment was a Hewlett-Packard Series HP 1100 equipped with a diode array detector. The stationary phase was a C18 LiChrospher 100 analytical column (250 mmx 4 mm i.d.) with a particle size of 5 mm thermostated at 30°C. The flow rate was 1 mL/min and the absorbance changes were monitored at 280 nm. The mobile phases for chromatographic analysis were: (a) acetic acid/water (2.5:97.5) and (b) acetonitrile. A linear gradient was run from 95% (a) and 5% (b) to 75% (a) and 25% (b)

| Table 2. Selenium, vitamin E (α-tocopherol acetate) and oleuropein contents of the diets. |
| Selenium, mg/kg | Oleuropein, mg/kg | α-tocopherol acetate, mg/kg |
|------------------|------------------|-----------------------------|
| NC               | 0.21             | -                           | 89.93                        |
| TA200            | 0.24             | -                           | 278.64                      |
| O200             | 0.22             | 195.72                      | 79.72                       |
| TA200+OSe        | 0.50             | -                           | 280.14                      |
| O200+OSe         | 0.48             | 198.02                      | 81.16                       |

NC, contained 0.15 mg/kg inorganic Se and 50 mg/kg α-tocopherol acetate (vitamin E); TA200, supplemented with 200 mg/kg α-tocopherol acetate to NC diet; O200, supplemented with 200 mg/kg oleuropein (olive leaf extract) to NC diet; TA200+OSe, supplemented with 200 mg/kg α-tocopherol acetate and 0.3 mg/kg organic Se to NC diet; O200+OSe, supplemented with 200 mg/kg oleuropein and 0.3 mg/kg organic Se to NC diet.

| Table 3. The effects of dietary treatments on body weight, body weight gain, feed intake and feed conversion ratio of quails reared under thermoneutral and heat stress. |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                   | BW, g           | BWG, g          | FI, g            | FCR, g           |
| DT                | 21 days         | 28 days         | 35 days         | 21 to 35 days   | 21 to 35 days   | 21 to 35 days   |
| NC                | 98.02           | 137.30          | 162.97          | 106.89           | 441.48          | 4.13            |
| NS                | 91.87           | 128.79          | 150.18          | 95.29            | 434.10          | 4.55            |
| TA200             | 100.55          | 137.16          | 164.03          | 110.39           | 463.31          | 4.20            |
| O200              | 98.37           | 133.16          | 166.59          | 112.33           | 459.79          | 4.10            |
| TA200+OSe         | 95.31           | 132.95          | 156.05          | 102.67           | 433.15          | 4.22            |
| O200+OSe          | 96.19           | 133.94          | 167.64          | 112.05           | 477.74          | 4.27            |
| NC                | 94.73           | 130.82          | 159.14          | 103.36           | 459.00          | 4.45            |
| NS                | 93.64           | 140.13          | 166.15          | 112.04           | 477.72          | 4.29            |
| TA200             | 90.29           | 127.60          | 161.56          | 103.36           | 459.00          | 4.45            |
| O200              | 91.87           | 133.87          | 163.86          | 108.00           | 468.36          | 4.37            |
| TA200+OSe         | 94.60           | 133.54          | 156.89          | 101.10           | 437.79          | 4.36            |
| O200+OSe          | 95.45           | 132.38          | 163.39          | 107.50           | 466.47          | 4.17            |
| NC                | 1.941           | 1.515           | 1.957           | 2.523            | 10.111          | 0.159           |
| NS                | 97.35           | 136.34          | 165.48          | 110.74           | 464.01          | 4.20            |
| TA200             | 93.88           | 130.58          | 157.70          | 102.19           | 441.90          | 4.35            |
| O200+OSe          | 1.227           | 0.957           | 1.237           | 1.595            | 6.395           | 0.100           |
| NC                | 0.019           | 0.786           | 0.028           | 0.048            | 0.039           | 0.492           |
| NS                | 0.037           | 0.000           | 0.016           | 0.001            | 0.011           | 0.012           |
| DT x TT           | 0.942           | 0.072           | 0.331           | 0.926            | 0.877           | 0.884           |

DT, dietary treatment; TT, temperature treatment; TN, thermoneutral temperature; HS, heat stress; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion rate; NC, diet containing 0.15 mg/kg inorganic Se and 50 mg/kg α-tocopherol acetate; O200, diet supplemented with 200 mg/kg oleuropein (olive leaf extract) to NC diet; TA200+OSe, diet supplemented with 200 mg/kg α-tocopherol acetate and 0.3 mg/kg organic Se to NC diet; O200+OSe, diet supplemented with 200 mg/kg oleuropein and 0.3 mg/kg organic Se to NC diet. α Values in the same column not sharing a common superscript differ significantly (*P<0.05; **P<0.01; ***P<0.001).
during 20 min.; it changed to 50% (a) and (b) in 20 min (40 min, total time); in 10 min it changed to 20% (a) and 80% (b) (50 min, total time), after re-equilibration in 10 min (60 min, total time) to initial composition. Oleuropein in OLE was identified by comparing its retention times with the corresponding standards. The average amount of oleuropein of OLE was 97.0 mg/kg OLE. With attention of oleuropein content of OLE, the OLE was supplemented to provide the 200 mg oleuropein/kg diet. Selenium concentrations of the experimental diets were analysed using hydride generation atomic fluorescence spectroscopy of an acid digest of the samples according the method described by Surai (2000). Selenium concentrations in the diet samples were calculated from the linear relationship (r²=0.999) obtained using sodium selenite as a standard. Vitamin E (α-tocopherol acetate) concentrations in the diets were determined using the HPLC system, after sample saponification with ethanolic potassium hydroxide (KOH) in the presence of pyrogallol (Surai et al., 1996). In short, triplicate samples of the diets (0.5 g) were mixed with 5 mL pyrogallol in ethanol (1:10 w/v) and 1.25 mL 60% potassium hydrox-ide, and then capped under nitrogen gas, vortexed and saponified at 70°C for 30 min. in a water bath. After these treatments, samples were cooled on ice and 7 mL 5% sodium chloride and 5 mL hexan were supplemented to the cool samples and vortexed. This mixture was left to stand on ice in the dark for 30 min and then the hexane phase including the lipophilic antioxidants was collected. Extraction using hexane was realized twice and the combined extracts were evaporated and the dry residue is re-dissolved in dichloromethane-methanol (1:1) ready for HPLC. Selenium, vitamin E (α-tocopherol acetate) and oleuropein contents of the experimental diets were given in Table 2.

### Measurements

**Performance parameters**

During the experimental period from 14 to 35 days, the growth performance of quails was evaluated by recording body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) on a weekly basis. Quails were weighed to ±0.1 g at hatch and 1 g thereafter. On the same day, FI was recorded to the nearest g and FCR was calculated weekly as the amount of feed consumed per unit of BWG. Mortality was recorded daily throughout the experiment.

**Haematological analysis**

At 35 days of age, 16 quails from each treatment group were randomly selected and bled from the brachial vein. Sodium pentobarbital injection (100 mg/kg) was used for anaesthesia to the experimental quails was applied before slaughtering. Blood samples were taken to the tubes containing EDTA for estimating the H/L ratio. The bleeding procedure was limited to 1 min or less to minimize the effects of handling stress. The blood samples were smeared on a glass slide for the determination of the H/L ratio. After drying, the smears were stained with May-Grünewald and Giemsa stains (Gross and Siegel, 1983). One-hundred leukocytes were counted on 1 slide of each quail using a light microscope at x 1000 magnification. The H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes.

**Hsp70 gene expression analysis**

At the end of the study (35 days), 16 quails

| DT  | TT  | Heterophil/lymphocyte ratio | Hsp70, ng/mL |
|-----|-----|-----------------------------|-------------|
| NC  | TN  | 0.505⁺                     | 4.310⁶      |
|     | HS  | 0.706⁺                     | 3.397⁴      |
| TA200 | TN  | 0.369⁺                     | 2.103⁵      |
|     | HS  | 0.403⁺                     | 1.023³      |
| O200 | TN  | 0.351⁺                     | 2.156⁴      |
|     | HS  | 0.404⁺                     | 16.665⁵     |
| TA200+OSe | TN  | 0.342⁺                    | 2.069⁵      |
|     | HS  | 0.377⁺                     | 16.169⁵     |
| O200+OSe | TN  | 0.343⁺                    | 0.877⁵      |
|     | HS  | 0.374⁺                     | 15.549⁵     |
| DT  | NC  | 0.605⁺                     | 19.142⁺     |
|     | TA200 | 0.356⁺                 | 9.119⁺      |
|     | O200  | 0.377⁺                | 9.410⁺      |
|     | TA200+OSe | 0.359⁺           | 9.563⁺      |
|     | O200+OSe | 0.359⁺            | 8.213⁺      |
| SEM | TT  | 0.038                      | 1.850       |
|     | TN  | 0.382₂                     | 2.303⁵      |
|     | HS  | 0.453⁺                     | 19.876⁺     |
| SEM |     | 0.024                      | 1.171       |
| P value | DT  | 0.000                      | 0.001       |
|     | TT  | 0.041                      | 0.000       |
|     | DT x TT | 0.023           | 0.016       |

DT, dietary treatment; TT, temperature treatment; TN, thermonuclear temperature; HS, heat stress; NC, diet containing 0.15 mg/kg inorganic 5e and 50 mg/kg α-tocopherol acetate (vitamin E); TA200, diet supplemented with 200 mg/kg α-tocopherol acetate to NC diet; O200, diet supplemented with 200 mg/kg oleuropein (olive leaf extract) to NC diet; TA200+OSe, diet supplemented with 200 mg/kg oleuropein and 0.3 mg/kg organic Se to NC diet. "⁺,"⁺⁺" and "⁺⁺⁺" show that columns' data are significantly different (*P<0.05; **P<0.01; ***P<0.001). "⁻" and "⁻⁻" show the interaction between dietary treatments and temperature treatments; "⁺⁺" and "⁺⁺⁺" show the interaction between temperature treatments and dietary treatments.
Results and discussion

Performance parameters

Average BW of quails at 14 days of age was 55.156±0.776 g and did not differ statistically among the treatment groups. Weekly mean BWs and BWGs, FIs and FCR of quails from 21 to 35 days were shown in Table 3.

As shown in Table 3, BWs of quails at 21 days were significantly influenced by the dietary treatment (DT) and the temperature treatment (TT) (P<0.05). While the highest BW was obtained by the TA200 diet, the lowest BW was found at feeding with the O200+OSe diet at 21 days (P<0.05). In addition, the heat-stressed quails had significantly lower BWs at 21 days than that of quails reared at TN (P<0.05). BWs of quails at 28 days were significantly affected by only TT and the quails exposed to HS had significantly lower BWs than that of quails reared at TN (P<0.001). BWs at 35 days and BWGs and FIs from 21 to 35 days of quails were significantly influenced by both DT and TT. Quails fed the TA200, O200, TA200+OSe and O200+OSe diets had significantly higher BWs at 35 days and also higher BWGs and FIs from 21 to 35 days than those of quails fed with the NC diet (P<0.05). In addition, BWs at 35 days (P<0.05) and BWGs (P<0.01) and FIs (P<0.05) of quails from 21 to 35 days were significantly reduced by HS compared to those of quails exposed to TN. Moreover, only HS significantly deteriorated FCR of quails from 21 to 35 days compared to that of quails reared at TN (P<0.05).

Our results related to BWs are in agreement with the findings of the previous researchers who reported that heat exposure significantly reduced the final BW of quails (Sahin et al., 2006, 2008; Tuzcu et al., 2008) or broiler chickens under HS. Our finding related to FI and the positive effects of the increased glucocorticoid concentration due to HS (Sahin et al., 2006, 2008) on FI of the heat-stressed quails. In addition, Tuzcu et al. (2008) and Sahin et al. (2006, 2008) reported that the dietary supplementation of the natural antioxidants such as epigallocatechin-3-gallate, lycopene and tomato powder significantly increased FI of quails reared under HS. On the other hand, Wang et al. (2008) showed that the dietary Forsythia suspensa extract supplement did not influence daily FI of broiler chickens under HS.

Blood heterophil/lymphocyte ratio and brain Hsp70 gene expression

The effects of dietary supplementation of TA or O alone or with organic Se on blood H/L ratio and brain Hsp70 gene expression in quails reared under TN and HS were summarized in Table 4. Blood H/L ratio of quails was influenced by DT (P<0.001) and TT (P<0.05). Heat stress significantly increased blood H/L ratio of quails compared to that of quails exposed to TN (P<0.05). Additionally, feeding of quails with the TA200, O200, TA200+OSe and O200+OSe diets significantly reduced their blood H/L ratio when compared to that of quails fed with the NC diet (P<0.001). Moreover, there is a significant interaction between DT and TT in terms of blood H/L ratio of quail (P<0.05). Irrespective of DT, HS significantly increased blood H/L ratio compared to that of quails exposed to TN (P<0.05). There is also a significant interaction between TT and DT in terms of blood H/L ratio of quails (P<0.05). The supplementation of TA200 and O200 alone or with OSe to the diets of quails reared under TN or HS significantly decreased their blood H/L ratio compared to that of quails fed the NC diet. According to these results, the most significant DTs were the dietary supplementation of TA200 and O200 without or with OSe (P<0.05).

This observation is in agreement with a previous study that the dietary supplementation of genistein and hesperidin alone or in combination could potentially ameliorate the HS in terms of blood H/L ratio of broilers (Kamboh et al., 2013). In addition, Srikhun et al. (2010) reported that the dietary supplementation of polyphenols extracted from tamarind seed coat at 200 mg/kg level significantly increased the lymphocyte level in the blood circulation of broilers exposed to chronic HS. Our results related to blood H/L ratio of quails in the pres-
ent study have shown that the dietary supplementation of TA200 and O200 with OSe due to their mutual synergistic effects may lead to counteract the deleterious effects of HS. Brain Hsp70 gene expression was influenced by DT (P<0.01) and TT (P<0.001). HS significantly increased brain Hsp70 gene expression compared to that of quails exposed to TN (P<0.001). Brain Hsp70 gene expression in quails fed with the TA200, O200, TA200+OSe and O200+OSe diets was lower than that of quails fed the NC diet (P<0.01). Moreover, feeding the quails with the O200+OSe diet was the most effective DT for brain Hsp70 gene expression. In addition, there is a significant interaction between DT and TT in terms of brain Hsp70 gene induction of quail (P<0.05). Irrespective of the DT, HS significantly decreased brain Hsp70 gene expression (P<0.05). There is also a significant interaction between TT and DT in terms of brain Hsp70 gene expression of quail (P<0.05). The dietary supplementation of TA200 and O200 alone or with OSe, especially O200+OSe significantly reduced brain Hsp70 gene expression in quails that were exposed to TN or HS (P<0.05). Our results concur with a study of heat-stressed Japanese quail that reported the vitamin C or E supplementation for downregulation of brain Hsp70 gene level (Sahin et al., 2009b). Likewise, Kamboh et al. (2013) pointed out that biomarker of HS including Hsp70 mRNA of breast muscle was significantly decreased by the dietary combined supplementation of genistein and hesperidin. In addition, our results related to Hsp70 gene expression are in accordance with the findings of Sahin et al. (2010) and Orhan et al. (2013) who reported that the supplementation of resveratrol and epigallocatechin-3-gallate to diet of heat-stressed quail significantly decreased liver Hsp70 expression. Our study demonstrated that the dietary supplementation of TA200 and O200 alone or with OSe may have function in the cellular antioxidant cascade to decrease ROS production/accumulation. On the other hand, the most effective dietary treatment for amelioration of HS has been informed. Brain Hsp70 gene expression was the O200+OSe diet. This situation can be explained by the inhibiting NF-κB expression and activating Nr2 expression that were activated and suppressed in HS of several antioxidant phytochemicals such as EGCG, resveratrol, curcumin and lycopene (Sahin et al., 2013). Enhanced expression of NF-κB in heat-stressed quails might be related to their activation and translocation to overcome oxidative stress in HS. Nr2 plays an important 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, haeme oxygenase-1, glutathione S-transferase and UDP-glucuronosyltransferase (Na and Surh, 2008).

Conclusions

In conclusion, the present study showed that dietary supplementation of oleuropein alone or with organic selenium eliminated similarly with those of quails fed diets supplemented with α-tocopherol acetate alone or with organic selenium the negative effects of heat stress on growth performance, blood H/L ratio and brain Hsp70 gene expression of Japanese quails.

References

Abidin, Z., Khatoon, A., 2013. Heat stress in poultry and the beneficial effects of ascorbic acid (vitamin C) supplementation during periods of heat stress. World’s Poultry Sci. Assoc. 69:135-152.

Altan, O., Altan, A., Cabuk, M., Bayraktar, H., 2008. Effects of heat stress on some blood parameters in broilers. Turk. J. Vet. Anim. Sci. 24:145-148.

Andreadou, I., Iliodromitis, E.K., Mikros, E., Constantinou, M., Agalis, A., Magiatis, P., Skaltsounis, L., Rambert, E., Tsantili-Kakoulidou, A., Th Kremastinos, D., 2006. The olive constituent oleuropein exhibits anti-ischemic antioxidative and hypolipidemic effects in anesthetized rabbits. J. Nutr. 136:2213-2219.

AOAC. 2007. Official methods of analysis, 18th ed. Association of Official Analytical Chemists, Washington, DC, USA.

Aruoma, O.I., Deiana, M., Jenner, A., Halliwell, B., Kaur, H., Banni, S., Corongiu, F.P., Dessi, M.A., Aeschbach, R., 1998. Effect of hydroxytyrosol found in extra virgin olive oil on oxidative DNA damage and on low-density lipoprotein oxidation. J. Agric. Food Chem. 46:5181-5187.

Benavente-Garcia, O., Castillo, J., Lorento, J., Oruto, A., Del Rio, J.A., 2000. Antioxidant activity of phenolics extracted from Olea europaea L. leaves. Food Chem. 68:457-462.

Chini, H., Cillard, J., Cillard, P., Rahmani, M., 1991. Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. J. Am. Oil Chem. Soc. 68:307-312.

Dridi, S., Decuyper, E., Buyse, J., 2013. Cerulein upregulates heat shock protein-70 gene expression in chicken muscle. Poultry Sci. 92:2745-2753.

Droge, W., 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82:47-95.

Duncan, D.B., 1955. Multiple range test and multiple F tests. Biometrics. 11:1-42.

Endgecombe, S.C., Stretch, G.L., Hayball, P.J., 2000. Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. J. Nutr. 130:2996-3002.

Gross, W.B., Siegel, H.S., 1983. Evaluation of the heterophyl/lumphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972-979.

Gu, X.H., Hao, Y., Wang, X.L., 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. Poultry Sci. 91:790-799.

Hayes, J.E., Allen, P., Brunton, N., O’Grady, M.N., Kerr, J.P., 2011. Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: olive leaf extract (olea europaea L) lutein, sesamol and ellagic acid. Food Chem. 126:948-955.

Hosseini-Mansouh, N., Chekani-Azar, S., Tehrani, A.A., Lotfi, A., Manesh, M.K., 2010. Influence of dietary vitamin E and zinc on performance, oxidative stability and some blood measures of broiler chickens reared under heat stress (35°C). J. Agribiol. 27:103-110.

Jemai, H., Bouaziz, M., Fki, I., El Feki, A., Sayadi, S., 2008. Hypollipidemic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. Chem. Biol. Int. 176:88-98.

Jena, B.P., Panda, N., Patra, R.C., Mishra, P.K., Behura, N.C., Panigrahi, B., 2013. Supplementation of vitamin E and C reduces oxidative stress in broiler breeder hens during summer. Food Nutr. Sci. 4:33-37.

Kamboh, A.A., Hang, S.Q., Bakhetgul, M., Zhu, W.Y., 2013. Effects of genistein and hesperidin on biomarkers of heat stress in broilers under persistent summer stress. Poultry Sci. 92:2411-2418.

Lako, J., Trencery, V.C., Wahliqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., Premier, R., 2007. Phytochemical flavonoids, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chem. 101:1727-1741.

Lara, L.J., Rostagno, M.H., 2013. Impact of heat
stress on poultry production. Animal 3:356-369.
Le Tutour, B., Guedon, D., 1992. Antioxidative activities of Olea europaea leaves and related phenolic compounds. Phytochemistry 31:1173-1178.
Lee, O.H., Lee, B.Y., 2010. Antioxidant and antimicrobial activities of individual and combined phenolics in olea europaea leaf extract. Biorecs. Techn. 101:3751-3754.
Lin, H., Decuyper, E., Buyse, J., 2006. Acute heat stress induces oxidative stress in broiler chickens. Comp. Biochem. Phys. A 144:11-17.
Mahmoud, K.Z., Edens, F.W., Eisen, E.J., Havenstein, G.B., 2004. Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers (Gallus gallus domesticus) subjected to cyclic heat stress. Comp. Biochem. Phys. B 137:35-42.
Na, H.K., Surh, Y.J., 2008. Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EOCG. Food Chem. Toxic. 46:1271-1278.
NRC, 1994. Nutrient requirements of domestic animals, 9th ed. National Academic Press, Washington, DC, USA.
Orhan, C., Tuzcu, M., Gencoglu, H., Sahin, N., Hayirli, A., Sahin, K., 2013. Epigallocatechin-3-gallate exerts protective effects against heat stress through modulating stress-response transcription factors in poultry. Brit. Poultry Sci. 54:447-453.
Özkan, S., Basmacıoğlu-Malayoğlu, H., Yalçın, S., Karadaş, F., Koçtürk, S., Çabuk, M., Oktay, G., Özdemir, S., Özdemir, E., Ergül, M., 2007. Dietary vitamin E (α-tocopherol acetate) and selenium supplementation from different sources: performance, ascites-related variables and antioxidant status in broilers reared at low and optimum temperatures. Brit. Poultry Sci. 48:580-593.
Pamok, S., Aengwanich, W., Komutrın, T., 2009. Adaptation to oxidative stress and impact of chronic oxidative stress on immunity in heat-stressed broilers. J. Therm. Biol. 34:353-357.
Ruiz-Gutierrez, V., Muriana, E.J.G., Maestro, R., Graciani, E., 1995. Oleuropein on lipid and fatty acid composition of rat heart. Nutr. Res. 15:37-51.
Ruiz-Gutierrez, V., Dela Puerta, R., Catala, A., 2001. The effect of tyrosol, hydroxytyrosol and oleuropein on the non-enzymatic lipid peroxidation of rat liver microsomes. Mol. Cell. Biochem. 217:35-41.
Sahin, K., Kucuk, O., 2001. Effects of vitamin E and selenium on performance, digestibility of nutrients and carcass characteristics of Japanese quails reared under heat stress (34°C). J. Anim. Physiol. An. N. 85:342-348.
Sahin, K., Sahin, N., Yaralioglu, S., Onderci, M., 2002. Protective role of supplemental vitamin E and selenium on lipid peroxidation, vitamin E, vitamin A, and some mineral concentrations of Japanese quails reared under heat stress. Biol. Trace Element Res. 85:59-70.
Sahin, K., Onderci, M., Sahin, N., Gursu, M.F., Khachik, F., Kucuk, O., 2006. Effects of lycopene supplementation on antioxidant status, oxidative stress, performance and carcass characteristics in heat-stressed Japanese quail. J. Therm. Biol. 31:307-312.
Sahin, K., Orhan, C., Smith, M.O., Sahin, N., 2013. Molecular targets of dietary phytochemicals for the alleviation of heat stress in poultry. World. Poultry Sci. J. 69:113-123.
Sahin, K., Orhan, C., Tuzcu, M., Ali, S., Sahin, N., Hayirli, A., 2010. Epigallocatechin-3-gallate prevents lipid peroxidation and enhances antioxidant defense system via modulating hepatic nuclear transcription factors in heat-stressed quails. Poultry Sci. 89:2251-2258.
Sahin, N., Orhan, C., Tuzcu, M., Sahin, K., Kucuk, O., 2008. The effects of tomato powder supplementation on performance and lipid peroxidation in quail. Poultry Sci. 87:276-283.
Sahin, K., Sahin, N., Kucuk, O., Hayirli, A., Prasad, A.S., 2009a. Role of dietary zinc in heat-stressed poultry: a review. Poultry Sci. 88:2176-2183.
Sahin, N., Tuzcu, M., Orhan, C., Onderci, M., Eroksuz, Y., Sahin, K., 2009b. The effects of vitamin C and E supplementation on heat shock protein 70 response of ovary and brain in heat-stressed quail. Brit. Poultry Sci. 50:259-265.
Shaila, S., Angshuman, S., Abhijeet, K., Samindranath, M., Pal Jayanta, K., 2005. Flufenoxuron, an acylurea insect growth regulator, alters development of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) by modulating levels of chitin, soluble protein content, and HSP70 and p34cdc2 in the larval tissues. Pestic. Biochem. Physiol. 85:84-90.
Silva, S., Gomes, L., Leitao, F., Coelho, A.V., Boas, L.V., 2006. Phenolic compounds and antioxidant activity of olea europaea L. fruits and leaves. Food Sci. Technol. Int. 12:385-396.
Srihun, T., Aangwanich, W., Kongbundit, W., 2010. Effects of polyphenols extracted from tamarind (Tamarindus indica L.) seed coat on body weight, white blood cells, bursa of fabricius and NDV-HI titer of broilers under chronic heat stress. Int. J. Poultry Sci. 9:988-995.
SPSSWIN, 2007. SPSS for Windows 6.1.4. SPSS WIN, Istanbul, Turkey.
Surai, P.F., Noble, R.C., Speake, B.K., 1996. Tissue-specific differences in antioxidant distribution and susceptibility to lipid peroxidation during development of the chick embryo. Biochim. Biophys. Acta. 1304:1-10.
Surai, P.F., 2000. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolks and the developing chick. Brit. Poultry Sci. 41:235-243.
Surai, P.F., 2002. Selenium. Natural antioxidant in avian nutrition and reproduction. Nottingham University Press, UK.
Tuzcu, M., Sahin, N., Karatepe, M., Cikim, G., Klinke, U., Sahin, K., 2008. Epigallocatechin-3-gallate supplementation can improve antioxidant status in stressed quail. Brit. Poultry Sci. 49:643-648.
Visioli, F., Gali, C., 1994. Oleuropein protects low density lipoprotein from oxidation. Life Sci. 55:1965-1971.
Visioli, F., Poli, A., Gali, C., 2002. Antioxidant and other biological activities of phenols from olives and olive oil. Med. Res. Rev. 22:65-75.
Wang, L., Piao, X.L., Kim, S.W., Piao, X.S., Shen, Y.B., Lee, H.S., 2008. Effects of Forsythia suspensa extract on growth performance, nutrient digestibility and antioxidant activities in broiler chickens under high ambient temperature. Poultry Sci. 87:1287-1294.
WPSC, 1989. European table of energy values for poultry feedstuffs, 3rd ed. WPSC Subcommittee Publ., Beekbergen, The Netherlands.
Zdunczyk, Z., Drabno, A., Jankowski, J., Juszkiewicz, J., Czech, A., Antoszkiewicz, Z., 2013. The effect of different dietary levels of vitamin E and selenium on antioxidant status and immunological markers in serum of laying hens. Polish J. Vet. Sci. 16:333-339.
Zulkifli, I., Al-Aql, A., Omar, A.R., Sazili, A.Q., Rajion, M.A., 2009. Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short or long tonic immobility reactions. Poultry Sci. 88:471-476.

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