The effects of dietary oleuropein and organic selenium supplementation in heat-stressed quails on tonic immobility duration and fluctuating asymmetry

Senay Sarica and Demir Ozdemir

Department of Animal Science, Gaziosmanpasa University, Tokat, Turkey; Vocational School in Technical Sciences, Akdeniz University, Antalya, Turkey

ABSTRACT

The current study aimed to compare the effects of the dietary supplementation of oleuropein (O) alone or with organic selenium (OSe) in combination with the dietary supplementation of α-tocopherol acetate alone (TA) or with OSe in combination on tonic immobility (TI) duration and fluctuating asymmetry in Japanese quails exposed to heat stress (HS) (34°C). A total of 800 two-week old quails were kept in wire cages in a temperature-controlled room at either 22°C (thermo-neutral, TN) or 34°C (HS) for 8 h/d and 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 the NC diet. HS increased TI (p < .001) and the lengths of the beak, outer toe, eye and nostril (p < .05) of quails. On the other hand, feeding diets containing TA200 alone or with OSe and O200 with OSe significantly shortened the TI duration and decreased the nostril length of quails exposed to HS compared to those of quails fed the NC and O200 diets (p < .05). It can be suggested that the TI and the asymmetry value are a sensitive indicator of thermal stress’ effect in quails. Moreover, the present study showed that especially the O200 + OSe diet may similarly alleviate the negative effects of HS on TI and mean bilateral asymmetry value of the nostril length in quail compared to the TA200 and TA200 + OSe diets.

ARTICLE HISTORY

Received 27 March 2017
Revised 29 June 2017
Accepted 3 July 2017

KEYWORDS

Fear; heat; natural antioxidants; quail; stress; well-being

INTRODUCTION

Heat stress (HS) due to the increased industrialisation and environmental degradation is a major problem of the poultry sector in the world (Ajakaiye et al. 2010). HS leads to huge economic losses in the poultry industry every year as a result of the increased mortality and decreased growth performance (Prieto and Campo 2010).

An animal loses its ability as emotion to learn to control and react to the environment when it learns that it cannot exert control over its environment. Poultry repeatedly subjected to the stress factors such as HS, oxidative stress, etc., can develop apathy due to their helplessness and be unresponsive (Veissier et al. 2012). Fear constitutes an important component of stress. Intense fear can dramatically decrease welfare and performance of birds. Tonic immobility (TI) has been defined as an adaptive psychophysiological response and as a measure of fearfulness and HS status in poultry, which is characterised by decreased responsiveness increased by physical restraint (Onbaşılar et al. 2007; Konca et al. 2009). Zulkifli et al. (2009) described the short and long TI of broilers is low-fear or high-fear response, respectively. The fearful birds show longer immobility reaction and longer TI (Altan et al. 2003). There are limited studies on the effects of HS on TI duration and results are inconsistent. Many studies reported that exposing birds to HS prolonged the duration of TI (Campo and Carnicer 1994; Yalcin et al. 2003; Konca et al. 2009; Zulkifli et al. 2009; Skomorucha et al. 2010) while TI duration was not influenced in broilers by HS (Zulkifli et al. 1999; Aksit et al. 2006). It is difficult to establish the degree of stress experienced by birds under practical conditions due to species, strain, degree of environmental temperature, humidity, diet, etc. Therefore, responses to dietary supplements and HS may be more variable in poultry.

There are some studies in literature about the effect of dietary supplementation of some feed additives on TI in poultry (Campo and Carnicer 1993; Jones 1996; Konca et al. 2009; Prieto and Campo 2010). Peral et al. (2014) showed that supplemental vitamin C and chromium had no significant effect on TI duration of...
broiler chickens. Mohamed et al. (2015) reported that dietary garlic (5%), onion (5%) and a mixture of garlic and onion (2.5% + 2.5%) to heat-stressed Japanese quails significantly decreased TI duration. In addition, Egguniwe et al. (2016) showed that betaine and its supplementation with ascorbic acid decreased TI duration compared to the control group. On the other hand, Zulkifli et al. (2000) and Zulkifli (2003) found that supplemental vitamin C dramatically reduced TI duration in stressed broilers.

A kind of bilateral asymmetry in morphological traits called fluctuating asymmetry (FA) also can be used as a biomarker of environmental and genetic stress and reflected health and chronic stress (Campo et al. 2000; Torrez-Terzo and Pagliosa 2007; Prieto and Campo 2010). There was a study that investigated the effect of HS on the FA of shank length, shank width and face length only in broiler chickens (Yalcin et al. 2003). Several antioxidants are used to alleviate the negative effects of HS.

Within this content, vitamin E and Se are the antioxidants in terms of reducing lipid peroxidation. The most active form of vitamin E is TA. TA protects the integrity of the cell membrane from lipid oxidation by means of being deposited in muscle tissues. Se also dependent glutathione peroxidase detoxified hydroperoxides, which maintained the work of vitamin E (Surai 2002). There are two forms such as inorganic and organic of Se used as supplement in the poultry diets. On the other hand, recently, the organic Se has been greatly used as an alternative to the inorganic Se (Zdunczyk et al. 2013). There are also some antioxidants as capsaicin, alicin, ascorbic acid (Lee et al. 2003; Whitehead and Keller 2003; Prieto and Campo 2010) among them for preventing the oxidative stress due to HS.

Moreover, in the last years, besides vitamin E-, flavonoids- and polyphenols-rich foods, such as olive-leaf and its extract have been coming into much prominence as natural antioxidants (Hayes et al. 2011). Oleuropein (O) is the most prominent and active phenolic compound that its concentration is 60–90 mg in dried olive leaves (Olea europaea L.)/g (Benavente-Garcia et al. 2000). The phenolic compounds found in the olive leaf or its extract (OLE) have indicated in vitro (Visioli and Gali 1994; Silva et al. 2006) and in vivo (Ruiz-Gutierrez et al. 1995; Endgecombe et al. 2000; Ruiz-Gutierrez et al. 2001; Andreadou et al. 2006; Jemai et al. 2008) antioxidant activities. Le Tutour and Guedon (1992) and Aruoma et al. (1998) reported that OLE and its phenolic compounds (O and hydroxytyrosol) have the more effective antioxidant activity than BHT or vitamin E. The antioxidant activities of the bioactive polyphenolic compounds in the olive leaves extract are partly associated with either donating hydrogen or electron, breaking the free radical chain reaction or preventing metal ion chelation (Lee and Lee 2010; Hayes et al. 2011). As far we know, the effects of the dietary supplementation of vitamin E or O with organic selenium (OSe) in combination on the FA and TI duration of heat-stressed quails have not been investigated yet.

The current study aimed to compare the effects of the dietary supplementation of O alone or with OSe in combination with the dietary supplementation of TA alone or with OSe in combination on TI duration and FA in Japanese quails exposed to HS (34°C).

Materials and methods

This study was conducted according to the guidelines of the Research Policy on Animal Ethics of the Gaziosmanpasa University. A total of 800 two-weeks-old male Japanese quails (Coturnix coturnix japonica) were used in this experiment. The study was conducted in accordance with animal welfare at the Poultry Research Centre of Gaziosmanpasa University. Quails in each wire cages were randomly allocated to 10 experimental groups, 4 replicates of 20 quails each in a 2 (temperature treatments) × 5 (dietary treatments) factorial arrangement from 3 to 5 weeks. Each cage has nipple drinkers and electrical heating system by thermostats and fan (F1 1453, Termobilim Resistance Industry Trade, Istanbul).

At 2 weeks of age, 5 of 10 experimental groups were subjected to either TN or HS) and the HS treatment room was divided from the TN temperature treatment room. Temperatures in these rooms were arranged as follows:

1. TN room: quails were kept at 24, 22 and 20 °C, at 3, 4 and 5 weeks, respectively. Relative humidity was 50%–60% during the experiment.
2. HS room: quails were exposed to 34 °C for 8 h/d (09:00–17:00 h) and then (from 17:00 to 09:00 h) to 24, 22 and 20 °C at 3, 4 and 5 weeks, respectively. Relative humidity was 60%–70% during the period of 2–5 weeks of age. Temperature and humidity in each room at two locations were monitored using a temperature-humidity record system (RTR-503). During the experiment, a fluorescent lighting schedule in each room was applied as 23 h light and 1 h dark and the average light intensity was 40 lux.
Quails in both temperatures were fed 1 of 5 different diets in mash form from 3 to 5 weeks of age. The 5 experimental diets were as follows: the basal diet (NC) containing 0.15 mg Se from sodium selenite/kg and 50 mg TA (vitamin E)/kg diet and the other diets were obtained by supplementing of 2 different natural antioxidant sources (TA or O) at 200 mg/kg level (TA200 and O200) or with organic Se in combination at 0.30 mg/kg level (TA200 + OSe and O200 + OSe) to the NC. The diets were formulated after feed ingredients were analysed for their crude protein (CP), ether extract, starch and total sugar according to the methods of the AOAC (2007). The NC (24% CP, 2900 kcal/kg ME) was formulated to meet minimum nutrient requirements established by the NRC (1994). The ingredients and calculated nutritional composition of the NC were given in Table 1. The TA was supplied from Kartal Chemistry Ltd. (Izmit, Turkey). Organic Se (OSe) was delivered as Selplex® 2000 from Alltech Biotechnology Food, Agricultural and Animal Products Company (Izmir, Turkey). Vitamin premix and trace mineral premix were prepared by Topkim–Topkapi Drug Company Ltd. (Istanbul/Turkey).

The OLE supplied by the Bio-Olive Ltd. Company (Ayvalik, Turkey) was filtered from Whatman No.1 filter paper. The filtrates were carried to a rotary evaporator to remove ethanol under reduced pressure at 38 °C, 120 rpm. The remaining aqueous solutions were lyophilised at −50 °C, 0.028 mbar. And then, the crude extracts were kept in vacuum bags at −80 °C until the use.

Total phenol content in the OLE and the experimental diets was analysed according to Folin–ciocalteu method of Lako et al. (2007). Results were expressed as milligram gallic acid equivalents (GAEs) per fresh weight and calculated as 196.81 ± 2.83 mg/GAEq/g extract.

The O contents of the OLE and experimental diets were analysed by HPLC at the Sciences of Izmir Institute of Technology (Izmir, Turkey). A Hewlett-Packard Series HP 1100 equipped with a diode array detector was used as the HPLC equipment. The O contents in OLE and experimental diets were found as 80.93, 278.64, 79.72, 280.14 and 81.16 mg/kg diet, respectively, for diets 1, 2, 3, 4 and 5.

TA contents of the diets were determined using the HPLC system. For this aim, samples were saponified by ethanolic KOH in the presence of pyrogallol according to the method of Surai et al. (1996). TA contents of the diets were established as 80.93, 278.64, 79.72, 280.14 and 81.16 mg/kg diet, respectively, for diets 1, 2, 3, 4 and 5.

A total of 120 quails (12 quails from each of 10 experimental groups) were tested for TI after their TN and HS exposure at 35 d of age at 11:00 a.m. Each quail was kindly taken hold with both hands, held in an upside down manner and transported uprightly to a separate room without eye contact with other quails. To determine TI, the quail was caught and placed on its back with the head hanging in a U-shaped wooden cradle in a few seconds (Jones and Faure 1981). The quail was restrained for 10 s. The experimenter sat about 1 m away of the quail and their eyes fixed on the quail to give the fear. If the chick remained inactive for 10 s after the experimenter removed their hands, a chronometer was started to record latency (s) until the quail righted itself. If the quail righted itself in less time than 10 s, it was considered that there is not any TI and the same procedure was repeated maximum 3 times. If the quail did not show a righting

| Ingredients, g/kg |  |
|------------------|---|
| Maize            | 400.7 |
| Wheat            | 100.9 |
| Soybean meal     | 345.8 |
| Full-fat soybean | 113.8 |
| Soybean oil      | 12.0 |
| Diacalcium phosphate | 8.5 |
| Limestone        | 12.2 |
| DL-methionine    | 0.2 |
| Sodium chloride  | 3.2 |
| Vitamin premixa  | 2.5 |
| Trace mineral premixa | 1.0 |
| Chemical composition (calculated) |  |
| 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 |
| Chemical composition (analysed) |  |
| 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 0.0375; α-tocopherol acetate 50 mg; menadione 5 mg; thiamine 3 mg; riboflavin 6 mg; pyridoxine 5 mg; cyancobalamin 0.03 mg; niacin 25 mg; calcium-D-pantothenate 12 mg; folic acid 1 mg; D-biotin 0.05 mg; apo-carotenonic acid ester 2.5 mg; choline chloride 400 mg.

Table 1. The ingredients and nutritional composition of the basal diet fed to quails from hatch to 5 weeks of age (as-fed basis).
response over the 10-min test period, a maximum score of 600 s was given for righting time. TI duration lasted from 0 to 600 s. Durations were logarithmically transformed before analysis (Prieto and Campo 2010).

The morphological traits were right (R) and left (L) beak length (distance between the corner of the mouth and top of the beak), face length, outer toe length (length of the fourth phalanx of the outer toe), back toe length (distance between tarsometatarsus and start of the nail of the back toe), eye length (distance between the corners of the eye), eye width, nostril length (distance between the outer points of the nostril) and nostril width. The inject sodium pentobarbital was applied to quails before slaughtering as anesthetics between right and left sides \([R – L]\). If a relationship between the mean values was positive, the asymmetry of a trait was calculated as the relative FA: \(2 \frac{|R – L|}{|R + L|}\). Relative FA was applied for all traits, which their distributions were not normal and transformed to arc-sin square root before analysis (Campo et al. 2000).

A 2 × 5 factorial analysis of the variance with a two-way ANOVA using the General Linear Model procedure of SPSS statistic package (SPSSWIN 2007) was applied to data related to TI duration and FA with a model including dietary treatments and temperature effects and their interactions. Significant differences between experimental treatments’ means were separated by Duncan’s multiple range test (Duncan 1955). Data were assumed to be statistically significant when \(p < .05\). Pearson’s chi-square analysis was performed for mortality rates.

**Results and discussion**

**Mortality**

No significant differences between the experimental treatments were observed for mortality \((p=.615)\).

**Tonic immobility**

The effects of the dietary supplementation of TA or O alone or with OSe on TI of quails subjected to TN and HS are summarised in Table 2. HS increased the duration of TI in quails compared to TN \((p<.05)\). This finding concurs with the results of Altan et al. (2003) and Yalcin et al. (2003) who pointed out that the exposure to HS of the birds increased the duration of TI. The heat-stressed quails tended to be more fearful than quails exposed to TN. This situation showed that response to a thermal stressor is associated with fear-related behaviour. HS may fail to activate the hypothalamus-pituitary-adreno-cortisol axis related to emotional arousal (Campo and Carnicer 1993; Skomorucha et al. 2010). As a result of this, HS increased fear responses of birds as evidenced by enhanced the TI duration. This result is not in agreement with those found by Zulkipli et al. (1999), Aşıt et al. (2006) and Prieto and Campo (2010) indicating that the TI

| Dietary treatment | Temperature treatment | Tonic immobility |
|-------------------|-----------------------|------------------|
| NC                | TN                    | \(^{A}2.93^b\)   |
|                   | HS                    | \(^{A}7.57^a\)   |
| TA200             | TN                    | \(^{A}4.90^b\)   |
|                   | HS                    | \(^{A}5.13^b\)   |
| O200              | TN                    | \(^{A}7.30^a\)   |
|                   | HS                    | \(^{A}4.88^b\)   |
| TA200 + OSe       | TN                    | \(^{A}5.00^a\)   |
|                   | HS                    | \(^{A}4.97^b\)   |
| O200 + OSe        | TN                    | \(^{A}6.30^b\)   |
|                   | HS                    | \(^{A}6.87^a\)   |
| DT                | NC                    | 65.25\(^a\)      |
|                   | TA200                 | 50.73\(^a\)      |
|                   | O200                  | 62.46\(^a\)      |
|                   | TA200 + OSe           | 49.07\(^a\)      |
|                   | O200 + OSe            | 59.23\(^a\)      |
| SEM               | TN\(^1\)              | 5.32\(^a\)       |
|                   | HS\(^2\)              | 64.19\(^a\)      |
| P-value           |                       | 0.0018           |
| DT                |                       | 0.0032           |
| TT                |                       | 0.0049           |
| DTxTT             |                       | 0.0018           |

\(^{A}\) The values in the same column not sharing a common superscript differ significantly \((p<.05)\).

\(^{A}\) The small letters in right show the interaction between DT and TT.

\(^{A}\) The capital letters in left show the interaction between TT and DT.

\(^{A}\) TN: thermonutral temperature;

\(^{A}\) HS: heat Stress; DT: dietary treatment; TT: temperature treatment.

\(^{A}\) Values in the same column not sharing a common superscript differ significantly \((p<.05)\).
duration of broilers was not influenced by HS. Moreover, the TA200, TA200 + OSe and O200 + OSe diets decreased the duration of TI in quails compared to that of quails fed the O200 and NC diets (p < .05). There is a significant interaction between dietary treatments (DTs) and temperature treatments (TTs) in terms of the TI duration in quails. Irrespective of DTs, HS increased the TI duration compared to that of quails exposed to TN (p < .05). There is also an interaction between TTs and DTs in terms of the TI duration of quails (p < .05). The TI duration of quails exposed to TN or HS was shortened by especially TA200 + OSe diet compared to those of quails fed the other diets (p < .05). This finding suggests that the effect of TA200 + OSe is greater in HS in others. In addition, feeding with TA200 and O200 + OSe diets also significantly shortened the TI duration of quails exposed to TN or HS compared to those of quails fed the O200 and NC diets (p < .05). Our results related to the TI duration are consistent with the findings of Jones (1996) who reported that the ascorbic acid supplementation shortened the duration of TI in laying hens. On the other hand, there are some studies where the diet-ary ascorbic acid supplementation did not affect the TI duration in male turkeys (Konca et al. 2009) and leghorn chicks (Campo and Carnicer 1993) exposed to HS.

Recently, studies about the new feed additives, such as, plant extracts that promote integrity, development and proper functioning and prevent from oxidative stress of the intestinal mucosa have been carried out. This is because the mucosa plays an important role in protecting the immune system and also synthesises about 80%–90% of the serotonin required for the proper function of the organism as well as its responsibility of nutrients digestion and absorption (Silva et al. 2012). The dietary supplementation of TA200 alone or with OSe or O200 with OSe might have been alleviated by HS and the oxidative stress due to HS on the birds and improved the intestinal flora. As a result, these DTs in our study might have caused to increase at the serotonin synthesis in the improved intestine and to reduce blood cortisol, providing a feeling of calmness and well-being to the quail (Silva et al. 2012). As a result of this, the birds become less active and less nervous (Mohamed et al. 2014).

### Asymmetry

The effects of the dietary supplementation of TA or O alone or with OSe on mean bilateral asymmetry values of various morphological traits of quails reared under TN and HS are given in Table 3. The lengths of face and back toe and the widths of eye and nostril of quails reared under TN and HS were not affected by the dietary supplementation of TA or O alone or with OSe. This finding concurs with that found by Yalcin et al. (2003) who pointed out that there are no

| Dietary treatment | Temperature treatment | Face length (mm) (DA) | Beak length (mm) (DA) | Outer toe length (mm) (FA) | Back toe length (mm) (DA) | Eye length (mm) (DA) | Eye width (mm) (DA) | Nostril length (mm) (FA) | Nostril width (mm) (FA) |
|-------------------|-----------------------|-----------------------|-----------------------|--------------------------|-------------------------|---------------------|---------------------|------------------------|------------------------|
| NC                | TN                    | 0.54                  | 0.12                  | 4.98                     | 0.68                    | 0.46                | 0.08                | 5.72                   | 9.49                   |
|                   | HS                    | 0.31                  | 0.28                  | 6.73                     | 0.12                    | 0.34                | 0.15                | 7.23                   | 8.71                   |
| TA200             | TN                    | 0.24                  | 0.15                  | 5.35                     | 0.30                    | 0.25                | 0.11                | 5.36                   | 7.30                   |
|                   | HS                    | 0.54                  | 0.23                  | 6.05                     | 0.38                    | 0.52                | 0.14                | 6.18                   | 12.33                  |
| O200             | TN                    | 0.25                  | -0.07                 | 5.08                     | 0.11                    | 0.57                | 0.14                | 5.80                   | 11.20                  |
|                   | HS                    | 0.56                  | 0.44                  | 6.48                     | 0.62                    | 0.18                | 0.09                | 7.52                   | 9.71                   |
| TA200 + OSe       | TN                    | 0.56                  | 0.27                  | 5.05                     | 0.44                    | 0.13                | 0.13                | 6.48                   | 7.83                   |
|                   | HS                    | 0.22                  | 0.03                  | 6.00                     | 0.27                    | 0.61                | 0.14                | 5.25                   | 11.15                  |
| O200 + OSe       | TN                    | 0.39                  | 0.13                  | 3.88                     | 0.23                    | 0.37                | 0.13                | 4.99                   | 12.28                  |
|                   | HS                    | 0.45                  | 0.16                  | 8.08                     | 0.47                    | 0.36                | 0.12                | 6.00                   | 8.05                   |
| DT                | NC                    | 0.43                  | 0.20                  | 5.90                     | 0.40                    | 0.40                | 0.12                | 6.50*                  | 9.1                    |
|                   | TA200                 | 0.39                  | 0.19                  | 5.70                     | 0.34                    | 0.39                | 0.12                | 5.80*                  | 9.8                    |
|                   | O200                  | 0.40                  | 0.19                  | 5.80                     | 0.36                    | 0.37                | 0.12                | 6.70*                  | 10.5                   |
|                   | TA200 + OSe           | 0.39                  | 0.15                  | 5.50                     | 0.35                    | 0.37                | 0.13                | 5.90*                  | 9.5                    |
|                   | O200 + OSe            | 0.42                  | 0.14                  | 6.00                     | 0.35                    | 0.37                | 0.12                | 5.50*                  | 10.2                   |
| SEM               |                       | 0.198                 | 0.166                 | 0.115                    | 0.127                   | 0.130               | 0.154               | 0.117                  | 0.137                  |
| TT                | TN                    | 0.39                  | 0.12*                 | 4.90*                    | 0.351                   | 0.358*              | 0.12                | 5.70*                  | 9.6                    |
|                   | HS                    | 0.42                  | 0.23*                 | 6.70*                    | 0.371                   | 0.401*              | 0.13                | 6.40*                  | 10.0                   |
| SEM               |                       | 0.08                  | 0.11                  | 0.15                     | 0.08                    | 0.08               | 0.10                | 0.07                   | 0.09                   |

* = Values in the same column not sharing a common superscript differ significantly (*p < .05).

1 TN: thermoneutral temperature; 2 HS: heat stress; DT: dietary treatment; TT: temperature treatment; SEM: standard error of the mean.
significant differences between broilers exposed to TN or HS in terms of the relative asymmetry of face length. HS increased the lengths of beak, outer toe, eye and nostril of quails compared to those of quails exposed to TN ($p < .05$). On the other hand, DTs did not significantly affect the lengths of face, beak, outer and back toe, eye and the widths of eye and nostril. Our results related to the lengths of outer toe and back toe are agreement with the finding of Campo et al. (2000) who reported that there were no significant differences among groups fed the diets supplemented with capsaicin, alicin or ascorbic acid for the relative asymmetry of the length of leghorn chickens.

Feeding TA200, TA200 + OSe and O200 + OSe diets significantly decreased the nostril length of quails compared to those of quails fed the NC and O200 diets ($p < .05$). In addition, HS significantly increased the nostril length of quails compared to that of quails exposed to TN ($p < .05$). Respiratory rate is arranged by the respiratory tract. The nasal cavity is a heat exchanger and therefore helps rid the body of excess heat through evaporative cooling. Increasing respiratory rate from the nasal and the increase of nostril length might have been increased to the most obvious clinical sign of HS in poultry.

We found the significant association between temperature treatments and asymmetry because the mean asymmetry values for the lengths of beak, outer toe, eye and nostril in heat-stressed quails were significantly higher than those of quails exposed to TN. As a result, it can be suggested that the asymmetry value is a sensitive indicator of thermal stress effect in quails.

No effect of O200 was found in terms of the reduction of the duration of TI and nostril length. This result showed that dietary O200 supplementation alone may be enough to alleviate the negative effects of HS in terms of TI duration and nostril length of quails. Contrary to our findings, Oke et al. (2017) reported that the bioactive compound in OLE may have also enhanced the ability of the birds to cope with thermal stress during the hot dry season.

Conclusions

In conclusion, HS increased the lengths of beak, outer toe, eye and nostril of quails compared to those of quails exposed to TN. On the other hand, feeding diets containing TA200 alone or with OSe and O200 with OSe significantly shortened the TI duration and decreased the nostril length of quails exposed to HS compared to those of quails fed the NC and O200 diets. As a result, it can be suggested that the TI and the asymmetry value are a sensitive indicator of thermal stress’ effect in quails. Moreover, the present study showed that the dietary supplementation of O with OSe may alleviate the negative effects of HS on TI and mean bilateral asymmetry of the nostril length in quail compared to the dietary supplementation of TA alone or with OSe.

Acknowledgements

The authors conducted the research, designed, did the statistical analyses and wrote the paper. Finally, the author commented on early and final versions of the manuscript.

Disclosure statement

The authors have declared that there is no conflict of interests.

Funding

This project was supported by a grant from The Scientific Research Project Coordinating Office of Gaziosmanpasa University (Project No: 2010/56).

References

Ajakaiye JJ, Perez-Bello A, Mollineda-Trujillo A. 2010. Impact of vitamins C and E dietary supplementation on leukocyte profile of layer hens exposed to high ambient temperature and humidity. Acta Vet Brno. 79:377–383.

Aksit M, Yalcın S, Ozkan S, Metin K, Özdemir D. 2006. Effects of temperature during rearing and crating on stress parameters and meat quality of broilers. Poult Sci. 85:1867–1874.

Altan O, Pabuccuoglu A, Altan A, Konyalioğlu S, Bayraktar H. 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Br Poult Sci. 44:545–550.

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

AOAC. 2007. Association of Official Analytical Chemists. 18th ed. Gaithersburg (MD); W.D.C.

Aruoma OI, Deiana M, Jenner A, Halliwell B, Kaur H, Banni S, Corongiu FP, Dessi MA, 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, Lorenzo J, Ortuno A, Del Rio JA. 2000. Antioxidant activity of phenolics extracted from Olea europaea L. leaves. Food Chem. 68:457–462.

Campo JL, Carnicer C. 1993. Realized heritability of tonic immobility in White Leghorn hens: a replicated single generation test. Poult Sci. 72:2193–2199.
Mohamed RA, Elazab MFA, El-Habashi NM, Elsabagh MR, El Tutour B, Guedon D. 1992. Antioxidative activities of individual and combined phenolics in leaves and related phenolic compounds. Food Sci Technol Int. 12:60–65.

Endgecombe SC, Stretch GL, Hayball PJ. 2000. Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. J Nutr. 130:2996–3002.

Hayes JE, Allen P, Brunton N, O’Grady MN, Kerry JP. 2011. Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: olive leaf extract (Olea europaea L.) lutein, sesamol and eagic acid. Food Chem. 126:948–955.

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

Jones RB, Faure JM. 1981. Sex and strain comparisons of the effects of betaine and ascorbic acid on tonic immobility, superoxide dismutase and glutathione peroxidase in broiler chickens during the hot dry season. J Vet Behav. 12:60–65.

Prieto MT, Campo JL. 2010. Effect of heat and several additives related to stress levels on fluctuating asymmetry, heterophil:lymphocyte ratio, and tonic immobility duration in White Leghorn chicks. Poult Sci. 89:2071–2077.

Ruiz-Gutierrez V, Muriana FJG, Maestro R, Graciani E. 1995. Oleuropein on lipid and fatty acid composition of rat heart. Nutr Res. 15:37–51.

Skomorucha I, Ewa S, Muchacka R. 2010. Effect of thermal stress on the developing chick embryo. Nutr Res. 15:235–243.

Silva JDT, Matos A, Sa, Hada FH, Gravena RA, Marques RH, Moraes VMB. 2012. Symbiotic and natural extracts in diets for Japanese quails at laying period. Ciencia Anim Bras. 13:1–7.

Oke OE, Emeshili UK, Iyasere OS, Abioja MO, Daramola JO, Ladokun AO, Abiona JA, Williams TJ, Rahman SA, Rotimi SO, et al. 2017. Physiological responses and performance of broiler chickens offered olive leaf extract under a hot humid tropical climate. J Appl Poult Res. doi: 10.3382/japr/pfx005.

Onbaslar EE, Erol H, Cantekin Z, Kaya Ü. 2007. Influence of intermittent lighting on broiler performance, incidence of tibial dyschondroplasia, tonic immobility, some blood parameters and antibody production. Asian-Austr J Anim Sci. 20:550–555.

Egbuniwe IC, Ayo JO, Kawu MU, Mohammed A. 2016. Effects of supplemental vitamin C and chromium on metabolic and hormonal responses, antioxidant status and tonic immobility reactions of transported broiler chickens. Biol Trace Elem Res. 157:224–233.

Perai AH, Kermanshahi H, Moghaddam HN, Zarban A. 2014. Effects of betaine and ascorbic acid on tonic immobility, superoxide dismutase and glutathione peroxidase in broiler chickens during the hot dry season. J Vet Behav. 12:60–65.

Duncan DB. 1955. Multiple range test and multiple F tests. Biometrics. 11:1–42.

Jones JL, Garcia Gil MG, Munoz I, Alonso M. 2000. Effects of several stressors on tonic immobility reaction of chickens. Arch Geflügel. 58:75–78.

Campo JL, Carnicer C. 1994. Effects of several stressors on tonic immobility reaction of chickens. Arch Geflügel. 58:75–78.

Campo JL, Garcia Gil MG, Munoz I, Alonso M. 2000. Relationships between bilateral asymmetry and tonic immobility reaction or heterophil to lymphocyte ratio in five breeds of chickens. Poult Sci. 79:453–459.

Duncan DB. 1955. Multiple range test and multiple F tests. Biometrics. 11:1–42.

Egbuniwe IC, Ayo JO, Kawu MU, Mohammed A. 2016. Effects of betaine and ascorbic acid on tonic immobility, superoxide dismutase and glutathione peroxidase in broiler chickens during the hot dry season. J Vet Behav. 12:60–65.
study with *Avicennia schaueriana* Stapf & Leechm. ex Moldenke (Acanthaceae). Insula. 36:75–94.

Veissier I, Aubert A, Boissy A. 2012. Animal welfare: A result of animal background and perception of its environment. Anim Front. 2:7–15.

Visioli F, Gali C. 1994. Oleuropein protects low density lipoprotein from oxidation. Life Sci. 55:1965–1971.

Yalcın S, Ozkan S, Cabuk M, Siegel PB. 2003. Criteria for evaluating husbandry practices to alleviate heat stress in broilers. J Appl Poult Res. 12:382–388.

Whitehead CC, Keller T. 2003. An update on ascorbic acid in poultry. World’s Poult Sci J. 59:161–184.

Zdunczyk Z, Drazbo A, Jankowski J, Juskiewicz 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. Pol J Vet Sci. 16:333–339.

Zulkifli I, Dass RT, Che Norma MT. 1999. Acute heat-stress effects on physiology and fear-related behaviour in red jungle fowl and domestic fowl. Can J Anim Sci. 79:165–170.

Zulkifli I, Che Norma MT, Chong CH, Loh TC. 2000. Heterophil to lymphocyte ratio and tonic immobility reactions to pre-slaughter handling in broiler chickens treated with ascorbic acid. Poult Sci. 79:402–406.

Zulkifli I. 2003. Effects of early age feed restriction and dietary ascorbic acid on heterophil/lymphocyte and tonic immobility reactions of transported broiler chickens. Asian Aust J Anim Sci. 16:1545–1549.

Zulkifli I, Al-Aqil A, Omar AR, Sazili AQ, Rajion MA. 2009. Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short or long tonic immobility reactions. Poult Sci. 88:471–476.