Influence of dietary genistein and polyunsaturated fatty acids on lipid peroxidation and fatty acid composition of meat in quail exposed to heat stress

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Abstract
This experiment was conducted to investigate the effects of polyunsaturated fatty acids (PUFA) and genistein on performance and meat fatty acid profiles in quail exposed to heat stress. A total of 360 Japanese quail were divided into 12 groups in a 2 × 2 × 3 factorial design; each group comprised 30 quail with five replicates and were kept either at 22 ± 2 °C for 24 h/day (Thermoneutral, TN) or 34 ± 2 °C for 8 h/day (08:00 to 17:00 h) followed by 22 °C for 16 h (heat stress, HS) conditions. The diet contained either two levels of PUFA at 15 or 45% of total fat or three levels of genistein at 0, 400, or 800 mg/kg. Bodyweight gain, feed intake, and feed efficiency were lower (p ≥ 0.01) for quail reared under heat stress and fed low PUFA. Increasing dietary genistein in a linear manner improved the productive performance (p < 0.001). Heat stress caused increases in serum and thigh meat malondialdehyde (MDA) concentrations and decreases in genistein and vitamin E and A concentrations in serum and thigh meat (p < 0.001). High PUFA (PUFA45) in the diet of quail caused greater 18:2, 18:3 ALA, EPA, DHA, n-6, and n-3 PUFA as well as total PUFA and total USFA percentages (p < 0.001) in the thigh muscle, some of which decreased with heat stress (p ≥ 0.006) with no regard to genistein supplementation. This study revealed that genistein with greater doses along with greater PUFA inclusion to the diet of quail reared under heat stress is recommended for alleviating adverse effects of heat stress and for yielding healthier meat for human consumption.

Keywords Heat stress · Genistein · Polyunsaturated fatty acids · Lipid oxidation · Quail

Introduction
Heat stress (HS) is one of the major concerns in poultry rearing. High surrounding temperature negatively influences the live performance, product quality, health status, survival, and general welfare of the poultry (Alfthan et al. 2015; Attia et al. 2016; Goel 2021; Nawab et al. 2018). Management strategies including ventilation and more appropriate floor space (Ratriyanto and Prastowo 2019) as well as the usage of specific nutrients in the diet such as probiotics, prebiotics (Li et al. 2020a, 2020b; Sohail et al. 2011), vitamins, and minerals (Sahin and Kucuk 2003; Sahin et al. 2009; El-Kholy et al. 2017; Orhan et al. 2019) in helping alleviate the adverse effects of heat stress have been widely appreciated.

Supplementing fatty acids (FA) to the diet of poultry enhances the growth, production performance, and health of poultry (Alagawany et al. 2019; Shang et al. 2004). Moreover, supplementing polyunsaturated fatty acids (PUFA) to the poultry diet has been reported to increase the deposition of PUFA in poultry products, and consequently, upon consumption, offer an important source of PUFA for humans (Konieczka et al. 2017; Wu et al. 2019). Consumption required amounts of PUFA as eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and alphalinolenic acid (ALA; 18:3n-3) is linked to the prevention of many chronic diseases, including cardiovascular problems and cancer (Manson et al. 2019; Mika et al. 2020).

As the main soy isoflavonoid, genistein plays an important role against oxidative stress and certain diseases such as diabetes and cancer (Gupta et al. 2015; Sanaei et al. 2018; Ustundag et al. 2007). It has also been reported that dietary supplementation of genistein to quail reared under HS
showed a protective effect against oxidative stress (Onderci et al. 2004). There is no study conducted in the literature using a combination of PUFA and genistein in poultry under heat stress. Therefore, the objective of the present work was to investigate the effects of dietary supplementation of different doses of PUFA and genistein on productive performance, serum and meat genistein concentrations, and anti-oxidant status, as well as meat fatty acid profiles in quail reared under heat stress.

**Materials and methods**

**Animals, diet, and experimental design**

The experiment was conducted following animal welfare regulations at the Veterinary Control and Research Institute of Elazig, Turkey, with all procedures approved by the Institutional Animal Care and Use Committee (02.05/04–15).

A total of 360 10-day-old Japanese quail (*Coturnix coturnix japonica*) were randomly divided into 12 groups in a 2 × 2 × 3 factorial design (2 environmental temperatures × 2 levels of dietary PUFA × 3 levels of dietary genistein), with each group containing 30 birds subdivided into five replicates with six birds each. The birds were kept in a temperature-controlled room at either 22 ± 2 °C for 24 h/day (thermonutral, TN) or 34 ± 2 °C for 8 h/day (08.00 to 17.00 h) followed by 22 °C for 16 h (heat stress, HS) during the experimental period (from day 10 to day 45). Animals were fed a basal diet (Table 1) or basal diet supplemented with two PUFA levels at 15 or 45% of total fat (g/100 g fat) or three genistein levels at 0, 400, or 800 mg/kg. The basal diet contained a 9% added fat source as either tallow only or a combination of tallow (ELET Inc. Elazig, Turkey), linseed oil, and fish oil (Oz-Gida Inc. Elazig, Turkey). The PUFA levels as 15 or 45% (g/g fat) for PUFA15 and PUFA45 in the diets were formulated as reported by Cortinas et al. (2004). The fatty acid compositions of the PUFA15 and PUFA45 in the diets are presented in Table 2. Genistein contained 98% aglycone form genistein and 2% starches as a carrier (Bonistein, DSM Nutritional Products, Istanbul, Turkey). Feed and water were offered ad libitum throughout the experiment.

The treatments used in this study were justified according to the literature. The treatment levels were determined based on the previous research published in the literature in a way that heat stress studies were usually conducted at thermoneutral (22 ± 2 °C) and high temperature (34 ± 2 °C for 8 h/day) to get relevant responses from birds. The levels of PUFA were determined based on the basal diet supplemented with low or high PUFA with 15 or 45% (g/g fat) as reported by Cortinas et al. (2004). Three levels of dietary genistein at 0, 400, or 800 mg/kg were also tested according to Onderci et al. 2004 and Sahin et al. 2006 using the same doses of 400 and 800 mg/kg in heat-stressed quails.

| Table 1 | Ingredients and nutrient contents of the basal diet fed to quail, dry matter basis |
|---------|-------------------------------------------------------------------------|
| Ingredient | % |
| Wheat | 39.47 |
| Soybean meal, 48% CP | 34.01 |
| Barley | 13.30 |
| Added oil | 9.00 |
| Dicalcium phosphate | 2.00 |
| Calcium carbonate | 1.0 |
| Vitamin-mineral mix | 0.50 |
| Sodium chloride | 0.40 |
| DL-methionine | 0.28 |
| L-lysine | 0.04 |
| Nutrient analyses | |
| ME, kcal/kg | 3200 |
| Crude protein, % | 22.96 |
| Ether extract, % | 10.75 |
| Crude fiber, % | 3.61 |
| Ash content, % | 5.83 |
| Genistein, mg/kg | 107.26 |

- The basal diet contained 9% added fat with different sources as either 90 g tallow/kg diet as PUFA15 or a combination of 35 g tallow/kg diet, 45 g linseed oil/kg diet, and 10 g fish oil/kg diet as PUFA45.
- Vitamin premix provides the following per kilogram: all-trans-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-rac-α-tocopherol acetate, 1.25 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; pyridoxine, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B12, 0.02 mg; folic acid, 0.55 mg; d-biotin, 0.1 mg. Mineral premix provides the following per kilogram: Mn (from MnO), 40 mg; Fe (from FeSO4), 12.5 mg; Zn (from ZnO), 25 mg; Cu (from CuSO4), 3.5 mg; I (from KI), 0.3 mg; Se (from NaSe), 0.15 mg; choline chloride, 175 mg.
- Analyzed by HPLC.
Table 2 Fatty acid profiles (g/100 g total fat) of PUFA15 and PUFA45 of diets

| Fatty acid | PUFA15 | PUFA45 |
|------------|--------|--------|
| C10:0      | 0.05   | 0.02   |
| C14:0      | 2.78   | 1.82   |
| C15:0      | 0.45   | 0.23   |
| C16:0      | 24.29  | 15.51  |
| C17:0      | 1.21   | 0.54   |
| C18:0      | 14.94  | 7.81   |
| C20:0      | 0.12   | 0.17   |
| Total SFA  | 43.84  | 26.10  |
| C16:1n-9   | 0.20   | 0.12   |
| C16:1n-7   | 2.30   | 1.68   |
| C18:1n-9   | 36.35  | 23.99  |
| C18:1n-7   | 1.63   | 1.31   |
| C20:1      | 0.29   | 0.32   |
| C24:1      | 0.09   | 0.82   |
| Total MUFA | 40.86  | 28.24  |
| C18:2n-6   | 13.43  | 18.28  |
| C18:3n-3 (ALA) | 1.58  | 25.03  |
| C18:4      | 0.29   | 0.24   |
| C20:4n-6   | –      | 0.13   |
| C20:5n-3 (EPA) | –    | 1.80   |
| C22:6n-3 (DHA) | –   | 0.18   |
| Total PUFA | 15.30  | 45.66  |
| PUFA:SFA   | 0.35   | 1.75   |

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids, ALA alpha-linolenic acid, EPA eicosapentaenoic acid, DHA docosahexaenoic acid

−70 °C in the deep freezer (Hettich, Tuttlingen, Germany) until analyzed.

Analysis of fatty acids by gas chromatography

For the analysis of fatty acids, samples were extracted according to the method reported by Hara and Radin (1978). One gram of feed and left thigh samples were homogenized with 5 ml of hexane-isopropanol (3:2, v:v) and centrifuged, and the supernatant was taken. Fatty acid methyl esters were prepared in the hexane/isopropanol phase, and 5 ml of 2% methanolic sulfuric acid was added, mixed, and left to methylate in a 50 °C oven (Memmert, Germany). Then, 5 ml of 5% sodium chloride (NaCl, Merck, Darmstadt, Germany) was added. Fatty acid methyl esters were extracted with hexane, and the hexane phase was treated with 5 ml of 2% potassium bicarbonate (KHCO₃, Merck, Darmstadt, Germany). The supernatant was taken and evaporated. The residue was dissolved with 1 ml of hexane and analyzed by gas chromatography (Shimadzu GC 17, Japan). The column temperature program was set from 120 to 220 °C. Fatty acid peaks were recorded and integrated using a Hewlett-Packard 3396 integrator. Calculations were made using the GC Solution (LabSolution GC solution 2.3) package program. Fatty acids of muscle were reported as a percentage (%).

Analysis of malondialdehyde, vitamins, and genistein by HPLC

Tissue and serum MDA, vitamin, and genistein levels were performed by using an HPLC (Shimadzu, Japan) with an ultraviolet (UV) detector (SPD-20A), pump (LC-20 AD), automatic sampling device (SIL-20A), column oven (CTO-10ASVP) units, and C18 column (ODS-3, 5 μm, 4.6×250 mm, Inertsil, GL Sciences, Japan). LC Solution (LabSolution LCL solution Release 1.21) package program was used.

The malondialdehyde (MDA) and vitamin C levels of serum and thigh meat samples were determined as reported previously (Sahin et al. 2016). Briefly, 300 μl 0.5 M perchloric acid (HClO₄, Riedel, Seelze, Germany) was added to the 400-μl serum sample and centrifuged, and the supernatant was taken. For tissue analyses, 0.5 g of thigh meat samples was homogenized with 1 ml ultra-pure water, 100 μl butylhydroxytoluene (500 μg/ml; 2,6-di t-butyl-p-cresol, BHT), and 1 ml 0.5 M HClO₄. Then, samples were centrifuged at 5000 rpm and 4 °C for 10 min, and the supernatant was taken for HPLC analyses. HPLC analysis conditions included column oven temperature as 30 °C, mobile phase, 30 mM KH₂PO₄ and methanol (82.5:17.5, v/v, pH 3.6), flow rate 1 ml/min, and wavelength 250 nm.

To detect serum and tissue fat-soluble vitamins A and E levels, samples were analyzed as described by Barim and Karatepe (2010). Briefly, 500 μl of extraction solution was added to the 300-μl serum sample to precipitate its proteins and extracted with 300 μl of n-hexane (Riedel, Seelze, Germany) three times, and hexane phases were evaporated. The residue was dissolved with methanol. For tissue analyses, 2 ml of ultrapure water and 200 μl of BHT (500 μg/ml) were added to the 0.5-g sample and homogenized. Then, 3 ml of extraction solution was added and mixed, and 500 μl of n-hexane was added and centrifuged at 5000 rpm and 4 °C for 10 min, and the upper hexane phase was removed. The extraction with hexane was repeated three times. The residue was dissolved with 200 μl of methanol. Analysis conditions: column oven temperature was set as 40 °C, mobile phase flow rate 1 ml/min, and wavelengths 326 nm for vitamin A, and 296 nm for vitamin E.

Genistein concentrations in serum and tissue samples were determined as defined previously with minor modifications (D’Souza et al. 2005; Tacyildiz et al. 2010). Three hundred microliters of 0.2 M sodium acetate (pH 5.0, C₂H₃NaO₂, Sigma-Aldrich, St. Louis, MO) containing 3500 units of the β-glucuronidase enzyme (Sigma Chemical, St. Louis, MO) was added to 300-μl serum sample and 0.5 g tissue sample and
incubated for 6 h. Then, 4400 µl of 80% methanol was added, mixed, and sonicated. The samples were centrifuged, and the supernatant was taken and evaporated. The residue was dissolved in methanol. Genistein levels were measured by a linear gradient using the reverse-phase separation technique on the HPLC device. Mobile phases forming the linear gradient: A mixture of 0.1% acetic acid (Merck, Germany), 5% acetonitrile and 94.9% ultrapure water, A mobile phase, 0.1% acetic acid, and 99.9% acetonitrile mixture was prepared as mobile phase B. Linear gradient applied during measurement: Initially, it was 84% A mobile phase and 16% B mobile phase, and until the 15th min, the A mobile phase was adjusted to 30% and the B mobile phase was 70%, and continued at these rates until the 18th min. The analysis was continued until 25 min by setting the mobile phase ratios to be A 84% and B 16% at 20 min. Analysis conditions: column oven temperature was set as 40 °C, mobile phase flow rate 1 ml/min, and wavelength 260 nm.

**Statistical analysis**

Quail were assigned randomly to one of 12 treatments in a 2 × 3 × 2 factorial arrangement of 2 environmental temperatures (TN vs. HS), two dietary PUFA levels (15 vs. 45% of total fat), and three genistein levels (0, 400, and 800 mg/kg). Analysis of variance was conducted using the PROC MIXED (SAS 2004) in a complete randomized design. The linear model to test the effects of treatments on response variables was as follows:

\[ Y_{ijkl} = \mu + ET_i + GN_j + PUFA_k + (ET \times GN)_{ij} + (ET \times PUFA)_{ik} + (GN \times PUFA)_{jk} + (ET \times GN \times PUFA)_{ijk} + e_{ijkl}, \]

where \( Y_{ijkl} \) = response variable, \( \mu \) = population mean, \( ET_i \) = environmental temperature (i = TN and HS), \( PUFA_k \) = PUFA level (i = 15 and 45%), \( GN_j \) = genistein level (0, 400, and 800 mg/kg), and \( e_{ijkl} \) = residual error, \( N (0, 1) \).

Linear and quadratic responses to genistein levels were attained using the polynomial contrast option. The linear model included the time effect and its relevant interaction as repeated measures for the performance variable. Data were reported as mean ± SEM. A probability value less than 0.05 was considered significant.

**Results**

**Performance**

As designed, initial BW was similar (\( p \geq 0.08; \) Table 3) among quail groups reared under TN or HS and supplemented with various levels of PUFA or genistein levels. However, final BW, BW gain, cumulative feed intake, and FCR were greater (\( p < 0.001 \)) for quail reared under TN compared with those reared under HS. Adding greater amount of PUFA (PUFA45) to the diet of quail resulted in lower productive performance measured as final BW, BW gain, cumulative feed intake, and FCR (\( p \geq 0.01 \)), whereas higher amounts of genistein caused greater final BW, weight gains, and FCR (\( p < 0.001 \)). Increases in dietary genistein levels resulted in a lower productive performance in quail reared under HS compared with those of quail reared at TN (\( p \geq 0.006, \) interaction). In addition, increases in dietary genistein levels resulted in a greater productive performance, except feed intake (\( p = 0.11 \)) in quail supplemented with low PUFA compared with those of quail supplemented with high PUFA (\( p < 0.001, \) interaction). Increased genistein levels in the diet caused positive linear responses in final BW, BW gain, and FCR in quail (\( p < 0.001 \)). There were no three-way interactions detected (\( p \geq 0.29 \)) for environmental temperature, PUFA, and genistein supplementations in quail for productive performances except initial BW (\( p < 0.04 \)).

**Thigh muscle fatty acid profile**

The quail reared under HS, compared with those of reared under TN, had greater 15:0, 16:0, 17:0, 18:0, and SFA percentages (\( p \geq 0.006; \) Table 4) along with lower 14:0, C16:1n-9, C18:1n-9, 18:2, 18:3 (ALA), 20:5 (EPA), 22:4, 22:6 (DHA), total PUFA, total MUFA, and total USFA as well as lower n-6 and n-3 PUFA percentages together with lower n-6 to n-3 PUFA ratios (\( p \geq 0.003 \)) in their thigh muscles. More PUFA (PUFA45) in the diet of quail caused greater 18:2, 18:3, 20:1, EPA, DHA, 24:1, n-6, and n-3 PUFA as well as total PUFA and total USFA percentages (\( p < 0.001 \)) along with lower 14:0, 14:1, 15:0, 16:0, C16:1n-9, C16:1n-7, 17:0, C18:1n-9, C18:1n-7, 18:4, 20:0, 20:1, 20:4, total MUFA, and total SFA percentages together with lower n-6 to n-3 PUFA ratios (\( p \geq 0.02 \)) in the thigh muscles. Adding genistein to the quail diet did not influence the FA profiles of muscles (\( p \geq 0.09 \)). Thigh muscle concentrations of 16:0 increased with 400 mg/kg genistein but decreased again with further increases in genistein at 800 mg/kg (\( p \geq 0.03; \) quadratic effect).

Thigh muscle concentrations of 16:0 and total SFA were greater in heat-stressed quail and stayed greater with the supplementation of greater PUFA (PUFA15) (\( p < 0.001; \) ET × PUFA interaction). However, thigh muscle concentrations of 18:0 were greater in heat-stressed quail and stayed greater with the supplementation of greater PUFA (PUFA45) (\( p < 0.001; \) ET × PUFA interaction). Quail reared under HS had lower thigh muscle concentrations of 18:2, ALA, EPA, 22:4, total PUFA, total USFA, total n-6 PUFA, and total n-3 PUFA when supplemented with greater PUFA (PUFA45) (\( p \geq 0.013; \) ET × PUFA interaction). In addition, the ratios of n-6 to n-3 were lower in quail reared in HS and stayed even lower with greater
PUFA supplementation (PUFA45) ($p < 0.008$; ET $\times$ PUFA interaction). Other two-way (PUFA $\times$ GN) or three-way interactions (ET $\times$ PUFA $\times$ GN) were not significant ($p > 0.05$).

**Serum MDA, genistein, and vitamin concentrations**

Quail reared under HS had greater ($p < 0.001$) serum MDA concentration but lower serum genistein, vitamin C, vitamin E, and vitamin A concentrations compared with those of quail reared under TN ($p < 0.001$; Table 5). The presence of greater PUFA (PUFA45) in the diet of quail resulted in greater concentrations of MDA ($p < 0.001$) but lower genistein serum concentrations ($p < 0.04$) with no changes in serum concentrations of vitamin C, vitamin E, and vitamin A ($p \geq 0.19$). Increasing genistein supplementation to the diet of quail linearly decreased serum MDA ($p < 0.001$) while linearly increased serum genistein concentrations ($p < 0.001$).

Serum MDA concentrations were greater in quail reared under HS when greater PUFA (PUFA45) was included in the diet ($p < 0.001$; ET $\times$ PUFA interaction). Similarly, serum MDA concentrations were greater in quail reared under HS, but supplementing the increasing genistein levels resulted in linear decreases in serum MDA concentrations in quail reared under HS ($p < 0.001$; ET $\times$ GN interaction). Heat stress caused decreases in serum genistein concentrations, but supplementing increasing genistein levels to the quail diet resulted in linear increases in the serum of genistein concentrations ($p < 0.001$; ET $\times$ GN interaction) in quail reared under HS. Serum MDA concentrations increased with PUFA45 supplementation. However, increasing genistein supplementation linearly decreased serum MDA concentrations in quail supplemented with greater PUFA ($p < 0.001$; PUFA $\times$ GN interaction). No three-way interactions were detected ($p \geq 0.08$) for environmental temperature, PUFA, and genistein supplementation in quail for serum MDA, genistein, and vitamin concentrations.

**Thigh muscle MDA, genistein, and vitamin concentrations**

Heat stress caused increases in MDA but decreases in genistein, vitamin E, and vitamin A concentration in thigh muscle.
### Table 4 Effects of dietary supplementation of polyunsaturated fatty acid and genistein on thigh muscle fatty acid profile (g/100 g fat) in quail reared under different environmental temperatures (n=10)

| Fatty acid | TN (Genistein level) | HS (Genistein level) | SEM | P < |
|------------|----------------------|----------------------|-----|-----|
|            | PUFA15               | PUFA45               |     |     |
|            | 0 400 800            | 0 400 800            |     |     |
| C14:0      | 2.07 2.18 2.13       | 1.44 1.51 1.48       |     |     |
| C14:1*     | 0.51 0.51 0.49       | 0.25 0.25 0.25       |     |     |
| C15:0      | 0.33 0.33 0.33       | 0.23 0.23 0.23       |     |     |
| C16:0*     | 21.97 21.57 22.23    | 17.92 17.61 18.22    |     |     |
| C16:1n-9   | 5.03 4.53 4.47       | 3.22 3.55 3.31       |     |     |
| C16:1n-7   | 0.66 0.67 0.65       | 0.49 0.45 0.48       |     |     |
| C17:0      | 0.67 0.68 0.69       | 0.47 0.47 0.47       |     |     |
| C18:0*     | 8.15 8.03 8.38       | 6.84 7.37 7.00       |     |     |
| C18:1n-9   | 44.37 45.11 44.60    | 30.79 29.67 29.57    |     |     |
| C18:1n-7   | 2.66 2.77 2.34       | 1.52 1.52 1.65       |     |     |
| C18:2n-6*  | 10.27 10.20 10.19    | 13.99 14.26 13.99    |     |     |
| C18:3n-3 (ALA)* | 1.41 1.42 1.48 | 18.92 19.30 19.81 |     |     |
| C18:4n-3   | 0.36 0.36 0.36       | 0.14 0.14 0.14       |     |     |
| C20:0      | 0.09 0.12 0.12       | 0.07 0.07 0.07       |     |     |
| C20:1      | 0.42 0.38 0.41       | 0.26 0.26 0.25       |     |     |
| C20:2n-6   | 0.09 0.09 0.09       | 0.09 0.09 0.09       |     |     |
| C20:3n-6   | 0.10 0.12 0.11       | 0.11 0.11 0.11       |     |     |
| C20:4n-6   | 0.49 0.55 0.56       | 0.40 0.38 0.41       |     |     |
| C20:5n-3 (EPA)* | 0.05 0.05 0.05 | 1.33 1.27 1.29 |     |     |
| C22:4n-6*  | 0.04 0.05 0.04       | 0.02 0.02 0.02       |     |     |
| C22:6n-3 (DHA) | 0.14 0.14 0.14 | 0.74 0.71 0.77 |     |     |
| C24:1      | 0.13 0.14 0.14       | 0.77 0.77 0.77       |     |     |
| Total MUFA* | 12.95 12.97 13.03    | 35.73 36.28 36.63    |     |     |
| Total USFA* | 66.72 67.09 66.12    | 73.02 72.74 72.92    |     |     |
| Total SFA*  | 33.28 32.91 33.88    | 26.98 27.26 27.08    |     |     |

**Note:**
- ET environmental temperature, TN thermoneutral, HS heat stress, GN genistein level, ALA alpha-linolenic acid, EPA eicosapentaenoic acid, DHA docosahexaenoic acid, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids, USFA unsaturated fatty acids, SFA saturated fatty acids
- Quadratic effect of supplemental genistein, p < 0.05
- ET x PUFA interactions, p < 0.05

Increasing genistein supplementation caused linear decreases in thigh muscle MDA but linear increases in genistein concentrations (p < 0.001). In response to increasing genistein supplementation to the diet of quail resulted in greater thigh muscle MDA concentrations (p < 0.001). However, muscles (p < 0.001; Table 6). Greater PUFA (PUFA45) supplementation in quail reared under different environmental temperatures (n=10). However,
supplementation, the reduction in thigh muscle MDA concentration was greater, and the rate of increase in thigh muscle genistein concentration was less for quail reared under HS than those reared under TN ($p < 0.001$; PUFA × GN interaction). There were no other two-way interactions detected ($p \geq 0.15$) for ET × PUFA or PUFA × GN in quail for thigh muscle MDA, genistein, and vitamin concentrations. In addition, there were no three-way interactions detected ($p \geq 0.37$) for environmental temperature, PUFA, and genistein suppletions in quail for thigh muscle MDA, genistein, and vitamin concentrations.

**Discussion**

The negative impact of heat stress manifested itself in reducing feed intake and weight gains along with depressed feed efficiency in the quail of the present work. These results are parallel to the findings of Roushdy et al. (2020), who reported reduced feed intake, weight gain, and feed conversion ratios in broiler chickens exposed to 4 or 6 h of heat stress (40 °C) per day. Orhan et al. (2020) also reported that laying quail exposed to 8 h of high ambient temperature (34 °C) per day consumed less feed and had lower apparent total tract digestibility of DM, OM, and crude protein. Depressed digestibility of nutrients occurs due to intestinal injury with reduced heights of the villus along with widening crypt depths of the intestine in broilers (He et al. 2018). Song et al. (2014) also stated that broilers exposed to high environmental temperature had intestinal ischemia and reduced intestinal integrity, leading to lower digestibility. However, Habashy et al. (2017) found that broiler chicks reared under high ambient temperature (35 °C) consumed less protein and fat with no changes in ileal digestibility of these nutrients.

Fat supplementation to the diet of broilers under heat stress is a strategic way to reduce the adverse effects of heat stress due to the lower heat production upon digestion and metabolism of fat compared with those of carbohydrates and proteins (Smith et al. 1978). However, the type of supplemented fat is another factor in this respect, as illustrated in the present work. Greater PUFA supplementation negatively influenced the production parameters at the current work, and these results are in agreement with the results of Seifi et al. (2018), who reported greater weight gain and feed intake along with a better FCR in heat-stressed broilers (36 °C) fed more SFA sources (coconut oil and tallow) compared with those of more USFA sources (olive and soy oil).
The presence of genistein, particularly with greater doses in the diet of heat-stressed quail, positively influenced the production parameters at the present work. Improved feed intake, weight gain, and FCR were also reported in broilers (Kamboh et al. 2013) and quail (Onderci et al. 2004) reared under HS and supplemented with various levels of genistein. In accord with the productive performance parameters, increasing genistein supplementation also linearly decreased serum and thigh meat MDA concentrations in quail at the present work. Similar to the results of the current work, serum MDA concentrations were also found decreased in heat-stressed (34 °C) quail supplemented with 200, 400, or 800 mg of genistein per kg of diet (Onderci et al. 2004). These results were expected because genistein is a potent antioxidant (Kładna et al. 2016) which linearly increased in serum and thigh muscle upon supplementing increasing amounts in the diet. Heat stress caused lower genistein concentrations in both serum and thigh meat tissues. These results could imply that some of the genistein supplemented to heat-stressed quail were used in antioxidant, cytoprotective, anti-inflammatory, and antiapoptotic functions (Hegab et al. 2018; Xia et al. 2019), leaving less genistein amounts available to be stored in tissues. As shown in the present work, greater amounts of genistein consumed by genistein-supplemented poultry meat may serve as a protective agent against cancer, obesity, diabetes, Alzheimer’s, and many more health problems (Chen et al. 2020; Li et al. 2020a, b).

Thigh meat FA profiles of quail, whether reared under HS or TN, were generally reflections of those of supplemental FA profiles including 16:0, C18:1n-9, 18:2, ALA, EPA, DHA, and total PUFA at the present work with no regard to effects of genistein supplements. Similarly, dietary fatty acids accumulating in the tissues of poultry were also reported by other researchers (Cortinas et al. 2004; Ribeiro et al. 2013; Sirri et al. 2003).

Greater content of oleic acid (18:1n9) and arachidonic acid (20:4n-6) in thigh muscle of quail supplemented with a low PUFA level (PUFA15) is desirable since arachidonic acid is an essential FA, and oleic acid is known as a health FA involving in improving insulin sensitivity (Ryan et al. 2000), reducing LDL-cholesterol but elevating HDL-cholesterol (Damasceano et al. 2011; Estévez-González et al. 2010). However, 18:2, ALA, EPA, DHA, total PUFA, total USFA, total n-3 PUFA, and total n-6 PUFA contents were greater in thigh muscle of quail supplemented with a high PUFA level (PUFA45). Therefore, not all but most beneficial FA were present in thigh meat of high PUFA-supplemented

| ET   | PUFA level (%) | Genistein level (mg/kg) | MDA | Genistein | Vitamin E | Vitamin A |
|------|----------------|-------------------------|-----|-----------|-----------|-----------|
| TN   | 15             | 0                       | 0.46| 0.21      | 4.52      | 2.83      |
|      | 400            | 0.42                    | 0.50| 4.55      | 2.84      |
|      | 800            | 0.40                    | 0.79| 4.54      | 2.98      |
|      | 45             | 0                       | 0.56| 0.21      | 4.50      | 2.73      |
|      | 400            | 0.55                    | 0.49| 4.52      | 2.76      |
|      | 800            | 0.52                    | 0.78| 4.53      | 2.79      |
| HS   | 15             | 0                       | 1.21| 0.10      | 2.15      | 1.55      |
|      | 400            | 1.12                    | 0.31| 2.21      | 1.56      |
|      | 800            | 1.08                    | 0.47| 2.24      | 1.56      |
|      | 45             | 0                       | 1.39| 0.10      | 2.11      | 1.54      |
|      | 400            | 1.25                    | 0.29| 2.11      | 1.54      |
|      | 800            | 1.22                    | 0.46| 2.13      | 1.54      |

SEM  
ANOVA  
ET  
0.001  0.001  0.001  0.001  
PUFA  
0.001  0.001  0.001  0.001  
GN  
0.001  0.001  0.001  0.001  
ET × PUFA  
0.004  0.001  0.001  0.001  
ET × GN  
0.001  0.001  0.001  0.001  
PUFA × GN  
0.001  0.001  0.001  0.001  
ET × PUFA × GN  
0.001  0.001  0.001  0.001  

ET environmental temperature, TN thermoneutral, HS heat stress, PUFA polyunsaturated fatty acids, GN genistein level

*Linear effect of supplemental genistein, p < 0.001
quail. Increased concentrations of 18:2 and ALA in poultry products provide, upon consumption, essential FA to humans. Similarly, greater EPA and DHA contents found in thigh meat of high PUFA-supplemented quail provide, upon consumption, more intake of these FA for humans. n-3 FA, particularly EPA and DHA, are associated with preventing several health problems, including cardiovascular diseases, immunity, Alzheimer’s disease, and weight gain (Elagizi et al. 2021; Miles et al. 2021; Simopoulos 2008; Swanson et al. 2012). However, ALA, EPA, and DHA are not consumed with adequate amounts by most people, including Americans (Burnett et al. 2020). Therefore, increasing the amounts of these FA in poultry products is a good strategy to get the proper amounts shown in the present work. The low ratios of n-6 to n-3 PUFA have been linked to preventing certain diseases, including cancer, diabetes, and cardiovascular diseases (Simopoulos 2006; Urlić et al. 2020). The low ratios were also in accord with greater concentrations of 18:2, ALA, EPA, DHA, total PUFA, total USFA, total n-3 PUFA, and total n-6 PUFA in the thigh muscle of quail supplemented with high PUFA, supporting healthier poultry products for human consumption.

In an attempt to increase the PUFA content of chicken meat by supplementing a mix of fat sources, including fish oil, to the diet, the case at the present work should be evaluated with a precaution. Poultry meat rich in PUFA content is vulnerable to oxidation since USFA is the substrate for oxidation. The oxidation process causes alterations in flavor, color, and nutritional value negatively (Luna et al. 2010). Therefore, in this respect, the present work results regarding meat quality should be evaluated with a limitation.

The present work revealed that heat stress is a negative factor in the productive performance of quail. Although moderate PUFA supplementation supported a greater productive performance, high amounts of PUFA supplementation yielded healthier meat with greater concentrations of n-3 FA, including EPA and DHA. Genistein supplementation to quail provided greater productive performance and greater meat genistein concentrations with no influence on meat FA profile. In conclusion, genistein with greater doses along with greater PUFA inclusion to the diet of quail reared under heat stress is recommended for alleviating the adverse effects of heat stress and for yielding healthier meat for human consumption.

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Author contribution Cemal Orhan and Nurhan Sahin were involved in the conception and design; Cemal Orhan performed the experiments and analyzed the samples; Cemal Orhan and Nurhan Sahin performed the lab analyses; Cemal Orhan, Osman Kucuk, and Kazim Sahin drafted the manuscript; Osman Kucuk, Cemal Orhan, Nurhan Sahin, and Kazim Sahin wrote and revised the paper. All the authors read and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work. All authors reviewed the results and approved the final version of the manuscript.

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Data availability The data are available upon request.

Code availability The codes for analyses are available upon request.

Declarations

Ethics approval The experiment was conducted following animal welfare regulations at the Veterinary Control and Research Institute of Elazig, Turkey, with all procedures approved by the Institutional Animal Care and Use Committee.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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