Effects of catechin and copper or their combination in diet on productive performance, egg quality, egg shelf-life, plasma 8-OHdG concentrations and oxidative status in laying quail (Coturnix coturnix japonica)

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ABSTRACT
The aim of this study was to determine the effects of catechin and copper supplementations to quail diet on performance, egg quality and lipid peroxidation, plasma 8-hydroxydeoxyguanosine (8-OHdG) concentrations and oxidative status. In this study, 300 quails, 10-week old, were divided into 4 groups with 5 replicates. The dietary treatments were a control diet and a control diet supplemented with 3000 mg/kg of green tea catechins (Cat), 80 mg/kg of copper proteinate (CuP), or 3000 mg/kg of Cat + 80 mg/kg of CuP (combination; Cat + CuP) for 8 weeks. Plasma 8-OHdG and enzyme activities did not change. Egg weight and specific gravity of Cat group were lower than that of the control group (p < 0.001). Egg yolk colour increased by dietary catechin (p = 0.001). The MDA concentrations in egg yolk, plasma and tissue decreased by dietary catechin; and kidney MDA concentrations also decreased by all treatments (p < 0.05). Consequently, dietary catechin had a negative effect on egg-specific gravity and a positive effect on egg yolk colour without affecting internal egg quality parameters. However, dietary catechin and copper, without causing DNA damage, reduced lipid peroxidation in egg and organism and has a positive effect on egg lipid peroxidation.

Introduction
Flavonoids are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine, all have variable phenolic structures (Panche et al. 2016). These structures have many pharmacological effects, such as antioxidant, antimicrobial, anti-carcinogenic, hypo-allergic, hypoglycaemic and anti-inflammatory activity (Biswas et al. 2000; Kara and Güçlü 2012; Kara et al. 2016a; 2016b; 2016c; 2016d). Many of these effects are related to their antioxidant properties and are important in both human and animal nutrition (Mahmoud et al. 2013). Numerous studies have demonstrated that green tea or its polyphenols has been used as an ingredient or feed additive in animals such as laying hen, broiler and dog (Biswas and Wakita 2001; Uuganbayar et al. 2005; Kim and Yang 2010; Kara et al. 2016b). In previous studies, it was stated that catechin supplementation to poultry diet decreased the lipid peroxidation of the liver, blood and egg and increased the reproductive performance (fertility, hatchability and hatchability of fertile eggs), meat quality and the self-life of animal products (Kara et al. 2016b, 2016c). Besides, dietary catechin highly increased the excretion of Zn, Mg and Cu via the faces, but increased Se level in serum (Kara et al. 2016d) and it negatively affected external quality parameters of egg (Kara et al. 2016b). Yamane et al. (1999) reported that green tea extracts can increase egg quality in a short-term study; in addition to this, green tea powder used for laying hens had positive effects on egg quality traits in a long-term study (Biswas et al. 2000; Uuganbayar et al. 2005). A number of studies have demonstrated that green tea catechins have the potential to affect the absorption and metabolism of ions because flavonoids interact with a variety of metal ions (Kara et al. 2016b). On the other hand, when phenolic compounds are also used in the presence of copper ion, they can act as pro-oxidants (Mahmoud et al. 2013).

Copper (Cu) is an essential trace mineral for poultry which functions in numerous physiological processes, primarily as a cofactor or an integral part of many important enzymes including cytochrome c oxidase, tyrosinase, β-hydroxyphenyl pyruvate hydrodase, dopamine beta hydroxylase, lysyl oxidase, and Cu-zinc superoxide dismutase (Vallee and Falchuk 1993; Underwood and Suttle 1999). These are very important for some biological processes such as growth, development, maintenance and production. Copper also protects cells from free radical damage because it is a component of superoxide dismutase and enhances iron transport as part of ceruloplasmin (Güçlü et al. 2008; Ajuwon and Idoiwu 2010).

The National Research Council (1994) recommends that copper be added at a rate of 2.5 mg/kg to laying hen diets and at a rate of 8 mg/kg to broiler chicken diets. However, inorganic copper compounds are often fed to poultry at the levels of 100–250 mg/kg diet for growth stimulation and therapeutic purposes (Güçlü et al. 2011). Therefore, feeding high levels of...
copper to poultry will have a negative effect on certain characteristics of the ecological environment and animal products. Because of this concern, many countries have started decreasing the maximum dietary Cu level. For example, the Association of American Feed Control Officials (2008) also limits the maximum use of supplemental Cu to 185 mg/kg as Cu-citrate. Some countries have limited the maximum level in pig diets to 75–100 mg/kg (MFAFP 2009). Copper compounds, complexed with organic compounds to increase the effectiveness of copper in the organism, are used rather than inorganic forms (Güçlü et al. 2011). Trace elements that are in chelated or complex form may improve the bioavailability of minerals for pigs and poultry (Kim et al. 2011).

The hypothesis of the study presented is the pro-oxidative activity and can generate active oxygen species such as hydrogen peroxide antioxidant activity of catechin (Hoshino et al. 2000) can be synergistically effective with copper with antioxidant properties. Also, the effect of copper feeding at high level, which has a negative impact on certain characteristics of the animal products (Güçlü et al. 2008), can be eliminated by chelation with catechin. There is also a hypothesis that the negative effect of catechin on egg weight and shell quality (Kara et al. 2016c) can be prevented with copper. Therefore, this study was performed to determine the effects of Cat and CuP and their mixtures on live weight, egg production, egg weight, feed intake, feed efficiency and egg quality, egg lipid peroxidation, plasma 8-hydroxydeoxyguanosine (8-OHdG) concentrations and oxidative status.

Materials and methods

The catechin used in the present study, derived from green tea leaves (Palmaxi 500 mg), was purchased from Valentis-U.S.A. and was added to the diet in powder form. Valentis Laboratories display on the label that this commercial product includes min. 95% polyphenols, min. 75% catechins, min. 45% epigallocatechin-3-gallate, and max. 3% caffeine on the label. Copper as a mineral supplement was used a commercially product (Bioplex Copper, Alttech, Nicholasville, KY/U.S.A.).

The study was carried out after the approval (decision number: 11/125) of the local ethics committee on animal use. In this study, ten-weeks old, 300, Japanese quails (Coturnix coturnix japonica) were distributed by similar live weight to five replicates of 15 quails each. All female quails were in egg production. At the beginning of the study, the average egg production of quails in each group was 90.1% (average of 10 days adaptation period). The quail was fed with a basal diet (with 17.5% crude protein and 2822 kcal/kg metabolizable energy in dry matter) (Li et al. 2011) (the control group) or basal diet supplemented with either 3000 mg/kg green tea (Camellia sinensis) catechins (Cat), 80 mg/kg copper proteinate (CuP), or 3000 mg green tea catechins /kg + 80 mg copper proteinate (Cat + CuP) (treatment groups) for eight weeks. The quail was reared in experimental cages of 45 × 100 cm in size (300 cm²/quail), with a photoperiod of 17-h daily. The animals were given ad libitum feed and water. The laying quail was weighed individually at the beginning and the end of the study. Eggs were collected daily, weighed using a digital scale. Feed intake, daily egg production and egg weight were recorded. Egg mass per quail per day was calculated as laying percentage, multiplied by average daily egg weight. The feed efficiency (g/g) was calculated using egg mass and feed intake (Feed efficiency = feed intake, g/egg mass output, g). The dead animals in the study period were recorded daily.

Daily egg production was recorded in all groups every day at 11 o’clock. At the middle and end of the study, eggs were collected two days in a row and then the specific gravity of a whole egg (g/cm³) was determined by Archimedes’ method (Wells 1968). The weights of eggs (g) were determined after leaving them for 24 h at room temperature. Twenty-five eggs (5 eggs from each replicate) were collected from every group at the middle and end of the study. Eggs were collected for two consecutive days in the middle and finally for quality analysis. In total, 100 eggs (25 × 2 × 2) from each group were broken for quality analyses. Broken eggs for analyses were left for 10 min before measurement in order to minimize any changes that may occur. The egg albumen height, yolk height (Digimatic Height Gage HDS, Mitutoyo, Japan), diameter (Vernier Caliper, Mitutoyo, Japan), albumen length and width (ABS Digimatic Present Caliper, Mitutoyo, Japan) (mm × 10⁻²) of all eggs were measured and used to calculate the Haugh unit, yolk index and albumen index. Haugh unit, an indicator of egg albumen quality, was calculated using the following formula: Haugh unit = 100 × log (H + 7.57 − 1.7 × W₀.37), where H = albumen height (mm) and W = egg weight (g) (Haugh 1937). The colour of egg yolk was assessed using the Roche colour scale. Egg shell thickness (mm × 10⁻²) was measured by a micrometre (Dia1 Caliper Gage, Mitutoyo, Japan).

At the end of the study, blood samples were collected into silicone tubes from 10 female quails by cervical dislocation in each group. The blood samples were centrifuged at 3000 rpm at room temperature for 10 min with the aim of extracting plasma. Approximately 25 mg of the liver and kidney tissue was weighted into a 1.5 mL centrifuge tube. Radio immune precipitation assay (RIPA) buffer with protease inhibitors (250 µl) was added to tubes. The tissues were homogenized with a sonicator (Bandelin, Sonopuls HD 2070®, Germany). The tissue homogenates were centrifuged at 1600g for 10 min at 4°C and the resultant supernatants were used for samples.

Thiobarbituric acid reactive substance (TBARS) values of the liver and kidney supernatants and the plasma were determined using a TBARS assay kit (Cayman®, U.S.A., Cat. No 10090055). Initially, 100 µL of plasma, 100 µL of sodium dodecyl sulphate and 4 mL of premixed colour reagent (Cayman Chemical Co., Ann Arbor, MI) were added to a 5 mL vial. Next, the vials were placed in boiling water for 1 h. After boiling, they were placed in an ice bath and allowed to incubate for 10 min. Then they were centrifuged at 1600g for 10 min at 4°C. Shortly after centrifugation, the vials were allowed to stabilize to room temperature for 30 min. Finally, the absorbance values of the supernatants (150 µL, in duplicate) from each vial were read at 530 nm on an ELISA microplate reader (μQuant®, BioTek Instruments, Inc., U.S.A.) for the TBARS concentration of samples. The TBARS values were expressed as the MDA concentration of samples (mmol/L) and were determined using a standard curve.
The plasma superoxide dismutase (SOD) concentrations in plasma were determined using a SOD assay kit (Cayman®, U.S.A., Cat. No 706002). Initially, 200 µL of the diluted radical detector and 10 µL of standard were added SOD standard wells on the plate. Next, 200 µL of the diluted radical detector and 10 µL of sample were added in the sample wells. Initially, 20 µL of diluted xanthine oxidase was added in all the wells. The xanthine oxidase was added as quickly as possible. The plate with 96 wells was carefully shaken for a few seconds. Then, the plate was covered with the plate cover and was incubated on a shaker for 30 min at room temperature. The absorbance values of the samples were read at 440–460 nm using μQuant (Bio-Tek) ELISA reader for the SOD concentration (U/mL) and were determined using a standard curve.

The plasma glutathione peroxidase (GPx) enzyme activity was determined using a GPx assay kit (Cayman®, U.S.A., Cat. No 703102). Initially, 120 µL of assay buffer and 50 µL of co-substrate mixture and 20 µL of dilute GPx (control) were added to positive control wells. Then, 100 µL of assay buffer, 50 µL of co-substrate mixture and 20 µL of sample were added to sample wells. 20 µL of cumene hydroperoxide was added to all the wells and 20 µL of diluted GPx was added in all the wells. Next, 100 µL of assay buffer were added and were mixed in background or non-enzymatic wells. Next, 120 µL of assay buffer were added in all the wells. The absorbance values of the samples were read at 450 nm on an ELISA microplate reader and 50 µL of co-substrate mixture and 20 µL of standard co-substrate mixture and 20 µL of sample were added to sample wells. Initially, 50 µL of sample or standard was added to be determined. Then, 50 µL PBS was added to blank wells and 50 µL of standard co-substrate mixture and were added to sample wells. Initially, 50 µL of sample or standard was added to be determined. Then, 50 µL PBS was added to blank wells and 50 µL of standard co-substrate mixture and were added to sample wells. Initially, 50 µL of sample or standard was added to be determined. Then, 50 µL PBS was added to blank wells and 50 µL of standard co-substrate mixture and were added to sample wells. Additionally, 50 µL of co-substrate mixture and 20 µL of sample were added to sample wells. 20 µL of cumene hydroperoxide was added to all the wells and the plate was carefully shaken for a few seconds to mix. The absorbance values of the samples were read every minute at 340 nm using μQuant (Bio-Tek) ELISA reader to obtain at least 5 time points for the GPx activity (nmol/min/mL) and were determined using a standard curve.

The plasma 8-hydroxydeoxyguanosine (8-OHdG) concentrations were determined using 8-OHdG assay kit (Northwest Life Science Specialist and LLC, Washington, Code: NWK 8-OHdG 02). The plasma was ultrafiltrated (Millipore-Amicon UFC501096) before working (250 µL sample + 500 µL PBS buffer). Initially, 50 µL of sample or standard was added to be assayed. Then, 50 µL PBS was added to blank wells and 50 µL reconstituted primary antibody was added to each well to be assayed except blank wells. The plate was shaken and was covered with an adhesive strip, then incubated at 4°C overnight. The contents of wells were emptied and 250 µL working wash buffer was added per well. Then, the wash buffer was inverted and the sink was emptied, this step was repeated 3 times. The empty plate was added 100 µL of reconstituted secondary antibody per well. The plate was shaken and was covered with an adhesive strip, then incubated at room temperature for 1 h. The plate was emptied and was washed 3 times as before. Then, 100 µL of working TMB Substrate was added per well. The plate was shaken and incubated at room temperature for 15 min in the dark. 100 µL of stop solution was added per well and the absorbance values of the samples were read at 450 nm on an ELISA microplate reader (μQuant®, BioTek Instruments, Inc., U.S.A.) for the 8-OHdG concentration (ng/mL).

The effect of different incubation times (0, 30, 60 and 90 min) on egg yolk MDA concentrations was analysed according to the method of Kara et al. (2016a). After storage at +4°C for a week, 15 eggs from each group were analyzed for MDA analyses. The basal diet was milled through a 1 mm sieve (IKA-Werke, Staufen im Breisgau, Germany) for use in wet chemical analyses. After drying, the basal diet was dried in an oven (Binder, Tuttingen, Germany) for 24 h at 105°C and dry matter (DM) was calculated. The ash content was determined using a muffle furnace at 500 °C for 8 h (AOAC 1990; method 942.05). Nitrogen (N) content was determined using the Kjeldahl method. Crude protein (CP) was calculated as N × 6.25 (AOAC 1990; method 942.01). The diethy ether extract (EE) and crude cellulose (CC) levels were determined according to the methods (method 920.39 and 7.06–7.070) reported by the AOAC (1990). The ingredients and chemical composition of basal diet are shown in Table 1. The basal diet was milled through a 1 mm sieve (IKA-Werke, Staufen im Breisgau, Germany) for use in wet chemical analyses. After drying, the basal diet was dried in an oven (Binder, Tuttingen, Germany) for 24 h at 105°C and dry matter (DM) was calculated. The ash content was determined using a muffle furnace at 500 °C for 8 h (AOAC 1990; method 942.05). Nitrogen (N) content was determined using the Kjeldahl method. Crude protein (CP) was calculated as N × 6.25 (AOAC 1990; method 942.01). The diethy ether extract (EE) and crude cellulose (CC) levels were determined according to the methods (method 920.39 and 7.06–7.070) reported by the AOAC (1990). The ingredients and chemical composition of basal diet are shown in Table 1. Statistical significance, among performance and egg-blood chemical parameters of quail for Cat, CuP and Cat + CuP diets, was determined by one-way variance analysis. Data were analyzed by the following statistical model: Yi = μ + τi + ei, where Yi is the observation, μ is the overall mean, τi is the effect of the treatment (i=4), and ei is the residual error. Statistical analysis of data was done with SPSS 15.0 software. Significance was defined at P values of <0.05.

### Results

In this study, the highest and lowest live weights were in the CuP (243.50 ± 3.04 g) and Cat (229.81 ± 2.90 g) groups, respectively (p<0.05). The feed intake and egg production values of Cat, CuP and Cat + CuP groups were similar to those of the control group (p>0.05). Egg weight values in Cat and Cat + CuP groups were lower than that of the control group (p<0.05). The feed efficiency values, calculated from egg
Table 2. Effect of Cat and CuP supplementation to diet on the performance of laying quail (Mean ± SE).

| Parameters                        | Control group | Cat | CuP | Cat+CuP | p-value |
|----------------------------------|---------------|-----|-----|---------|---------|
| Egg weight, g/quail              |               |     |     |         |         |
| Initial live weight, g/quail     | 75            | 236.37 ± 3.18 | 236.72 ± 2.53 | 237.12 ± 2.33 | 237.26 ± 2.59 | 0.995 |
| Final live weight, g/quail       | 75            | 233.04 ± 3.86 | 229.81 ± 2.90 | 243.50 ± 3.04 | 240.09 ± 3.81 | 0.024 |
| Feed intake, g/day/quail         | 5             | 35.37 ± 0.35 | 34.19 ± 0.53 | 35.98 ± 1.05 | 37.87 ± 1.47 | 0.091 |
| Egg production, %                | 3786**        | 90.16 ± 2.30 | 89.00 ± 3.02 | 90.45 ± 0.89 | 89.89 ± 1.16 | 0.962 |
| Egg weight, g                    | 270           | 11.55 ± 0.06 | 11.36 ± 0.05 | 11.49 ± 0.05 | 11.23 ± 0.04 | 0.001 |
| Feed efficiency, g/g             | 3             | 3.44 ± 0.10 | 3.42 ± 0.07 | 3.42 ± 0.05 | 3.80 ± 0.11 | 0.023 |

Control group: fed with a diet did not supplement either catechins or copper; Cat group: fed with a diet supplemented 3000 mg/kg catechins; CuP group: fed with a diet supplemented 80 mg/kg copper as copper proteinate; Cat+CuP group: fed with a diet supplemented 3000 mg/kg catechins + 80 mg/kg copper as copper proteinate; SEM: Standard error of mean. *: Values are for each group, **: this value is for 90% egg production.

Note: Means in the same row which are superscripted differently are significantly different (Tukey Test).

Table 3. The effects of Cat and CuP supplementation to diet on internal and external egg quality in laying quail (total 100 eggs from every group at the middle (n = 50) and end (n = 50) of the study) (Mean ± SE).

| Egg quality parameters          | Control group | Cat            | CuP            | Cat+CuP | p-value |
|---------------------------------|---------------|----------------|----------------|---------|---------|
| Shell thickness, mmx10^-2       | 17.40 ± 0.20bc| 16.88 ± 0.25b  | 17.97 ± 0.18b  | 16.93 ± 0.20b | 0.002  |
| Specific gravity, g/cm^3        | 1.0665 ± 0.0013a | 1.0615 ± 0.0013b | 1.0681 ± 0.0010a | 1.0627 ± 0.0014b | 0.001  |
| Albumen index, %                | 10.20 ± 0.25  | 9.85 ± 0.34    | 9.64 ± 0.29    | 9.83 ± 0.25 | 0.462  |
| Yolk index, %                   | 43.88 ± 0.40b | 44.82 ± 0.66b  | 46.60 ± 0.76b  | 44.29 ± 0.34b | 0.002  |
| Haugh unit                      | 87.28 ± 0.52  | 86.06 ± 0.63   | 85.66 ± 0.56   | 86.11 ± 0.46 | 0.124  |
| Yolk colour                     | 9.50 ± 0.09b  | 10.50 ± 0.27b  | 9.40 ± 0.15b   | 9.44 ± 0.15b | 0.001  |

Control group: fed with a diet did not supplement either catechins or copper; Cat group: fed with a diet supplemented 3000 mg/kg catechins; CuP group: fed with a diet supplemented 80 mg/kg copper as copper proteinate; Cat+CuP group: fed with a diet supplemented 3000 mg/kg catechins + 80 mg/kg copper as copper proteinate; SE: Standard error of mean. Note: Means in the same row which are superscripted differently are significantly different (Tukey Test).

Table 4. The effects of Cat and CuP supplementation to diet on the MDA concentration (nmol/mg) of egg yolk in laying quail (n = 25) (Mean ± SEM).

| Incubation time | Control group | Cat | CuP | Cat+CuP | p-value |
|-----------------|---------------|-----|-----|---------|---------|
| 0 min           | 0.0399 ± 0.0066c | 0.0228 ± 0.0049c | 0.0247 ± 0.0049bc | 0.0208 ± 0.0020b | 0.031  |
| 30 min          | 0.0786 ± 0.0128a | 0.0467 ± 0.0055a  | 0.0437 ± 0.0039a  | 0.0485 ± 0.0057b  | 0.008  |
| 60 min          | 0.1206 ± 0.0083 | 0.1287 ± 0.0059 | 0.1319 ± 0.0039 | 0.1184 ± 0.0050 | 0.344  |
| 90 min          | 0.1708 ± 0.0145 | 0.1553 ± 0.0148 | 0.2165 ± 0.0500 | 0.1559 ± 0.0120 | 0.365  |

Control group: fed with a diet did not supplement either catechins or copper; Cat group: fed with a diet supplemented 3000 mg/kg catechins; CuP group: fed with a diet supplemented 80 mg/kg copper as copper proteinate; Cat+CuP group: fed with a diet supplemented 3000 mg/kg catechins + 80 mg/kg copper as copper proteinate; SE: Standard error of mean. Note: Means in the same row which are superscripted differently are significantly different (Tukey Test).

Discussion

In the study, final live weight was significantly increased in the CuP group. However, the Cat group’s final live weight value decreased significantly. In agreement with our result, Biswas and Wakita (2001) reported that green tea powder tended to decrease live weight gain with a higher dose. Also, the results in the study of Kim et al. (2011) reported that supplementation with 100 mg/kg of Cu as Cu-Methionine increased live weight gain. Jegede et al. (2015) also reported that diets, which contain 100 mg/kg Cu, had higher weight compared to those fed 50 and 150 mg/kg Cu. In addition, the positive effect of production, of Cat and CuP groups were the same as that of the control group (p < 0.05); but its Cat + CuP was higher than that of the control group (p > 0.05) (Table 2).

The CuP group had the highest shell thickness, while the Cat group had the lowest shell thickness (p < 0.05), and statistically significant differences between the treatment groups were found, but the differences among the treatment groups and the control group were not significant. In groups including catechin decreased egg-specific gravity (p = 0.001). The egg yolk index increased in the CuP group (p < 0.01). Dietary catechin and copper or their combination did not change the egg albumen index or Haugh unit (p > 0.05). The egg yolk colour was increased with catechin supplementation (p < 0.05) (Table 3).

The MDA concentration of egg yolk in laying quail was changed with incubation time and dietary supplements. In the Cat group, MDA concentration of egg yolk was decreased at 0 min of incubation (p < 0.05). But, MDA concentration of egg yolk decreased by all treatments groups at 30 min of incubation (p < 0.05). At incubation minutes after 30 min, MDA concentrations of egg yolk were increased, but did not change by dietary supplements (p > 0.05) (Table 4).

Plasma 80HdG, GPx and SOD concentrations did not change by supplemental of catechin, copper or their combination in laying quail diet (p > 0.05). The MDA concentrations in the plasma (p < 0.05) and the tissues of the liver (p < 0.05) and kidney (p < 0.05) were low with 3000 mg/kg supplementation of catechin to diet. In addition, MDA concentration in kidney tissue was also decreased by copper or catechin + copper in laying quail diet (p < 0.05) (Table 5).
Cu could be attributed to the bactericidal and/or other effects of Cu on the gastrointestinal tract microbiota.

With the removal of antibiotics from diets, studies are conducted on alternative additives that increase the yield performance, digestive tract modulator and animal product quality in poultry (Hajiafgahpor and RezaeiPour 2018). Copper has been a very popular feed additive in poultry diets because of its possible growth-promoting and antimicrobial effects. Some previous studies (Bakali et al. 1995; Skrivan et al. 2000), in which poultry diets had been supplemented with different sources and levels of organic and inorganic copper compounds, showed increased performance. In contrast, in the present study, supplementation of 100 mg/kg CuP had no effects on egg production, egg weight and feed efficiency or feed intake. These results confirmed the findings of researchers who found no significant influence of 150–450 mg/kg dietary copper as CuP on the feed intake, feed efficiency and egg weight in laying hen (Güçlü et al. 2008).

In the present study, the supplementation of 3 g/kg catechin to quail diet did not change egg production, feed intake and feed efficiency were parallel with the results of Kara et al. (2016c) used 2 or 4 g/kg catechin in laying quail diet. Kara et al. (2016d) showed that catechin supplementation to fattening quail diet did not have any effect on growth performance. Some study, which researched phenolic feedstuffs (green tea powder, dried grape pomace) as poultry feed supplement, did not change egg production, feed efficiency in laying poultry (Uuganbayar et al. 2005; Kojima and Yoshida 2008; Kara and Güçlü 2012). In addition, previous studies stated that the powder and extract of green tea increased performance in hen (Uuganbayar et al. 2005; Abdo et al. 2010).

In the present study, egg-specific gravity and egg shell thickness parameters in the Cat group were lower than those of the control and CuP groups. This effect could be attributed to the high content of polyphenols in the treatment diets which had a negative effect. In accordance with the findings of the study presented, Kara et al. (2016c) reported that 2 or 4 g/kg catechin supplementation reduced egg weight, egg-specific gravity and egg shell thickness in quail. In addition, previous studies demonstrated that supplementation of phenolic feedstuffs (dried green tea powder, dried black tea waste) at high rate in poultry decreased eggshell quality traits, such as eggshell thickness, eggshell strength and eggshell weight (Uuganbayar et al. 2005; Kojima and Yoshida 2008). The presence of polyphenolic compounds in the diet may have some adverse effects mainly associated with lower efficiency of nutrients, particularly protein and minerals, inhibition of digestive enzymes and increased excretion of endogenous protein (Kara and Güçlü 2012; Kara et al. 2016b; 2016d). In this study, Cu supplementation of increased egg yolk colour was similar to the results of Abdo et al. (2010). However, Kojima and Yoshida (2008) reported that green tea powder did not change egg yolk colour. Trace elements may affect eggshell quality by their catalytic properties as key enzymes by either involving in the process of membrane and eggshell formation or interacting directly with the calcite crystals in the forming eggshell. It was suggested that copper increased the activities of enzymes such as lysyl oxidase. This enzyme is a cuproenzyme and is involved in the conversion of lysine to cross-linked desmosine and isodesmosine (Chowdhury 1990). Consequently, Cu deficiency in hens results in abnormal eggshell formation. However, in the present study, the positive effect of CuP did not affect Haugh unit, yolk index and yolk colour score egg shell quality (eggshell thickness and egg-specific gravity). Similarly, Idowu et al. (2006) reported that laying diet supplemented with 125 and 250 mg/kg copper had no significant effects on egg shape index, shell index, specific gravity or shell thickness. In the presented study, the non-negative/positive effect of copper on egg shell quality was not observed in its combination with catechin.

Studies about the effects of supplementation with copper and catechin together on poultry performance were not found. In this study, the parameters of egg quality, such as egg albumen index, egg yolk index and Haugh unit and parameters of performance such as live weight, egg production and feed intake, were not affected by the combination of Cat + CuP. However, specific gravity, egg shell thickness and feed efficiency were affected negatively by this supplement.

In a previous study, it was stated that catechin intake was positively correlated with total antioxidant status concentrations and negatively correlated with plasma and tissue MDA concentrations in quail (Kara et al. 2016d). The decreases in plasma, tissue and egg MDA concentrations in this study may be associated with the transfer of catechin flavonoids to the blood stream or the increase of serum selenium, which are antioxidant mineral concentration (Kara et al. 2016b).

Kara et al. (2016b) indicated that 2 and 4 g/kg catechin supplementation to quail diet decreased the egg yolk MDA concentration. Besides, the present study results agree with previous studies which used green tea leaves powder/extracts and found reduced lipid peroxidation in the poultry egg (Uuganbayar et al. 2005; Abdo et al. 2010). The decreases in

### Table 5. The effects of Cat and CuP supplementation to diet on the plasma 8OHdG concentration and the tissue and the plasma lipid peroxidation in laying quail (n = 10) (Mean ± SE).

| Parameters       | Control group | Treatment groups | p-value |
|------------------|---------------|------------------|---------|
|                  | Plasma 8OHdG, ng/mL |                  |         |
|                  | 7.21 ± 0.44  | 7.12 ± 0.53      | 6.72 ± 0.37 | 7.23 ± 0.27 | 0.792 |
|                  | Plasma MDA, µmol/L | 2.79 ± 0.42² | 1.33 ± 0.34³ | 2.11 ± 0.23⁴ | 2.16 ± 0.33⁴ | 0.045 |
|                  | Liver MDA, µmol/L | 0.93 ± 0.16³ | 0.40 ± 0.08³ | 0.53 ± 0.10³ | 0.52 ± 0.09³ | 0.016 |
|                  | Kidney MDA, µmol/L | 2.70 ± 0.54³ | 1.57 ± 0.40³ | 1.12 ± 0.27³ | 0.67 ± 0.13³ | 0.002 |
|                  | Plasma SOD, U/mL | 4.91 ± 0.41 | 5.26 ± 0.62 | 4.06 ± 0.32 | 5.91 ± 0.72 | 0.145 |
|                  | Plasma GPx, nmol/mL | 3.44 ± 0.36 | 4.24 ± 0.29 | 3.35 ± 0.48 | 3.00 ± 0.40 | 0.186 |

Control group: fed with a diet did not supplement either catechins or copper; Cat group: fed with a diet supplemented 3000 mg/kg catechins; CuP group: fed with a diet supplemented 80 mg/kg copper as copper proteinate; Cat+CuP group: fed with a diet supplemented 3000 mg/kg catechins + 80 mg/kg copper as copper proteinate; SE: Standard error of mean. Note: Means in the same row which are superscripted differently are significantly different (Tukey Test).
tissue and plasma MDA levels may be related to (i) the catechin scavenging activity of free radicals; (ii) chelating with metal ions which are pro-oxidant; (iii) stimulation of the synthesis of endogenous antioxidant enzymes in cells; (iv) increasing of some minerals (such as selenium) in antioxidant defence system (Sutherland et al. 2006; Kara et al. 2016b; 2016c; 2016d).

The 8-OHdG, which is a critical biomarker of oxidative damage, permanently occurs to lipids of cellular membranes, proteins and DNA and carcinogenesis (Huang et al. 2015; Jiang et al. 2017). Huang et al. (2015) showed that heat exposure of broiler increased lipid peroxidation and 8-OHdG concentration in plasma. In another study, exposure to pentachlorophenol, which is an extensively used pesticide with toxicity, induced an increase in liver 8-OHdG concentration in quail (Jiang et al. 2017). In the present study, plasma 8-OHdG concentrations did not change by supplementation of catechin, copper or their combination in laying quail diet. This result demonstrated that suppletions of up to 3 g/kg catechin or 80 mg/kg copper to quail diet did not cause DNA damage or lipid peroxidation.

Endogenous antioxidant enzyme systems in cells, such as SOD and GPx, ensure protection against reactive oxygen species with enzymatic ways, and prevent DNA damage and protein oxidation. In the present study, dietary catechin and copper or their combinations did not change SOD concentration and GPx enzyme activity in plasma, but treatments decreased MDA concentration plasma, yolk and tissue, especially by only catechin supplementation.

Conclusions
As a result of the present study, feed intake, egg production, albumen index and Haugh unit did not change with dietary catechin, copper or their combinations. The dietary catechin in quail had a negative effect on egg-specific gravity and a positive effect on egg yolk colour, without affecting internal egg quality parameters. However, dietary catechin and copper, without causing DNA damage, reduced lipid peroxidation in egg and organism and has a positive effect on egg lipid peroxidation. These effects are more pronounced with only catechin using in diet. The negative effect of copper on egg-specific gravity could not be removed in its combination with copper.

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