The effects of propolis on serum malondialdehyde, fatty acids and some blood parameters in Japanese quail (Coturnix coturnix japonica) under high stocking density

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ABSTRACT

The objective of this study was to investigate the effects of dietary propolis supplementation of the basal diet on the fatty acids, serum malondialdehyde (MDA) and some blood parameters of Japanese quail feeding under high stocking density (HS). For that, 256 Japanese quail were divided into four treatment groups. Group I- HS fed on a basal diet, II- (HS-0.5) was fed on a basal diet supplemented with 0.5 g propolis/kg, III- (HS-1) was fed on a basal diet supplemented with 1 g propolis/kg, IV- (HS-1.5) was fed on a basal diet supplemented with 1.5 g/propolis/kg. MDA levels in serum (P < .01) were found to be significantly higher in the unsupplemented HS group. Globulin, total protein, low density lipoprotein cholesterol (P < .001) and alanine aminotransferase (P < .01) levels of unsupplemented HS group were found to be significantly higher. Propolis supplementation significantly affected the lipid composition of internal organs (kidney and liver) of HS-0.5, HS-1 and HS-1.5 groups by decreasing saturated fatty acid contents and by increasing polyunsaturated fatty acid contents. Consequently, propolis supplementation improved the quality of lipid composition of the internal organs of quail. It might be considered to prevent the negative effects of stocking density.

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1. Introduction

For a long time, fatty acids compositions in meat-producing animals have been attractive in terms of their implications for meat quality characteristic and for human health (Wood & Enser 1997; De Smet et al. 2004). Essential fatty acids are necessary for the maintenance of optimal health but they cannot be synthesized by the body and must be obtained from dietary sources (Singh 2005). Due to the beneficial effects of omega-3 polyunsaturated fatty acid (PUFA), people should consume this PUFA from animal products. Poultry meat as a source of animal proteins plays an important role in human diets (Sarıca 2003).

For many years, stocking density indicated the numbers of birds being reared in a given housing area (Thaxton et al. 2006). Breeding in crowded areas or reduction in cage floor area per animal may affect efficiency (Cravener et al. 1992; Ogan 1995). Comfortable areas for stocking density of quail are suggested as 150–210 cm²/quail (Faitarone et al. 2005; Narinc et al. 2013; Kucukonder et al. 2014). Stocking density is an environmental condition as temperature and humidity. Increased stocking density could be a cause for oxidative stress (Celik et al. 2010). Stocking density may enhance oxidative destruction and cause malondialdehyde (MDA) generation (Simsek et al. 2009). Also, it causes some detrimental effects on tissues. The negative effects of oxidative stress could be prevented with dietary supplemental natural and synthetic antioxidant substances (carotenoid, vitamins A, C and E, methionine, selenium, zinc, omega-3 fatty acids, flavonoids, coenzyme Q, lycopen, carnitine, some plant extracts and nutrigenomic additives) (Celik et al. 2010).

Propolis, incorporated into poultry diets may be an alternative. Propolis is one of the most important bee products (Kumova et al. 2002). It is an adhesive, dark yellow to brown coloured balsam that smells like resin. It is collected from the buds, leaves and similar parts of trees and such plants as pine, oak, eucalyptus, poplar and chestnut by bees and mixed with their wax (Ghisalberti 1979; Schmidt & Buchmann 1992; Krell 1996). Propolis has antioxidative (Miguel et al. 2014), antimicrobial (Akca et al. 2016), antifungal (Sariguzel et al. 2015), antiviral (Schnitzler et al. 2010) and antiinflammatory (Cavendish et al. 2015) effects. Antioxidative, antimutagenic, immunomodulatory and cytostatic effects of propolis are associated with its rich flavonoid, phenolic acid and terpenoid contents (Tiftik 1996; Prytzyk et al. 2003; Tatlı Seven 2008). Flavonoids are potent antioxidant, free radical scavengers and metal chelators. They prevent lipid peroxidation (Harborne & Williams 2000; Prytzyk et al. 2003; Wang et al. 2003). This study was aimed to determine the effects of propolis on serum MDA, fatty acids and some blood parameters (glucose, albumin, creatinin, globulin, total protein, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), low density lipoprotein (LDL) cholesterol in quail feeding under high stocking density (HS)).

2. Materials and methods

2.1. Animals and diets

Two-hundred and fifty six, eight-day-old Japanese quail were randomly divided into the unsupplemented group and the
three supplemented groups. They were placed in cages (40 cm × 32 cm) including four replicates from each group. Quail were reared in 80 cm²/quail cage densities (Das et al. 1990; Ozbay et al. 2004). The experimental groups were designed as no supplementing to basal ration under HS; supplementing of 0.5 g/kg propolis to basal ration under HS (HS-0.5); supplementing of 1 g/kg propolis to basal ration under HS (HS-1) and supplementing of 1.5 g/kg propolis to basal ration under HS (HS-1.5) for a period of 8–42 days. Corn and soybean meal-based feeds were formulated according to the requirements suggested by the NRC (1994). Diets were formulated as starter (until 21 d) and finishing diets (between 22 and 42 d). The ethanolic extraction (10%) of propolis (EEP) was prepared. For a 10% EEP solution 100 g of the ground propolis was added to 900 ml of 70% ethanol. Prior to analysis, the mixture was kept at room temperature in the dark. After extraction, extracts of propolis were filtered through Whatman No. 1 filter paper. Then the ethanolic extracted propolis was homogeneously mixed carefully to the basal diets. The ingredients and chemical composition of the diets are presented in Table 1. Chemical composition of feed ingredients (dry matter, crude protein (CP), ether extract, ash) as dried samples was analysed using AOAC (1995) procedures and crude fibre was determined by the methods of Crampton and Maynard (1970).

In the HS and HS-0.5, HS-1 and HS-1.5 groups, 16 quails were kept per pen (40 cm × 32 cm) over 42 days. Photoperiods of 23 h/d between 3–42 days were maintained. The temperature was maintained at 36° C for the first week. Thereafter, the temperature was reduced by three degrees each week to a minimum of 22 ± 2° C. Feed and water were given ad libitum. The body weights of the birds were measured individually on the 42nd day, 8 birds of similar body weight were selected from each treatment group and slaughtered. Blood samples were collected from the brachial vein of eight birds from each group. Serum was separated and stored at −20°C until analysis. Liver, kidney and abdominal fat tissue samples were taken immediately and stored at −20°C until analyses. They were thawed at +4°C and homogenized just before analysis.

### 2.2. Sample collection and analyses

Propolis samples were collected from the Karabük Province (Middle Black Sea). Hand-collected propolis samples were kept desiccated in the dark until the processing and extracted for a week with 100 ml of 70% ethanol at room temperature to obtain the extract. After filtration, the extract was evaporated using a vacuum evaporator at 45°C and then used in the experiment (Krell 1996).

Gas chromatography-mass spectrometry (GC-MS) was carried out to detect the main components of propolis by an Agilent GC 6890 gas chromatography coupled to an Agilent MSD 5973 mass detector under electron impact ionization. The main compounds of the propolis samples were identified and are listed in Table 2.

The levels of MDA were measured in the serum with the thiobarbituric acid reaction according to Placer et al. (1966). Thiobarbituric acid reacts with MDA, substrates similar to MDA and forms a stable pink color. The optical densities of MDA were measured at 532 nm using a spectrophotometer.

Some biochemical parameters of serum (glucose, albumin, creatinin, globulin, total protein, urea, AST, ALT, LDL cholesterol) were measured using an autoanalyser Olympus AU-600.

### 2.3. Extraction and analyses of fatty acids

Extraction of lipids from the tissue samples (kidney, liver and abdominal fat), experimental diets (starter and finishing) and propolis samples was performed according to the Har and Radin (1978) method. According to this method, 1 g of liver, kidney and abdominal fat tissues were broken down in a Micra-D.8 homogenizer for 1 min with 10 ml hexane isopropanol. The homogenized tissue was put into a centrifuge tube and was centrifuged at 4500 rpm for 10 min to separate the tissue pellet.

Fatty acid methyl esters were extracted according to the Christie (1992) methylation method. Fatty acids in the lipid extract were converted into methyl esters by 2% sulphuric acid (v/v) in methanol. Fatty acids were analysed in Shimadzu GC 17 ver. 3 gas chromatography. A 25 cm-long Machery Nagel (Germany) capillary column with a 0.25 µm internal diameter and a Permabond 25 µm film thickness was used in the analyses.

### 2.4. Statistical analysis

Data were subjected to analysis of variance. Differences between mean values were calculated by one-way analysis of variance and Duncan’s multiple range test (SPSS for Windows: 11.5 SPSS Inc. (2002)). The results were considered significant when ‘P’ values were less than .05.

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**Table 1. Components and chemical composition of the diets (g/kg).**

| Ingredients          | Starter       | Finishing    |
|----------------------|---------------|--------------|
| Maize                | 539.8         | 621.3        |
| Soybean meal (48%)   | 266.3         |              |
| Full fat soybean     | 124.8         | 276.5        |
| Poultry meal         | 25            | 60           |
| Soybean oil          | 9.4           | 14.3         |
| Limestone            | 11.9          | 2.9          |
| Bone meal            | -             | 16.5         |
| Dicalcium phosphate  | 11.9          |              |
| Salt                 | 3             | 2.1          |
| DL-methionine        | 2.8           | 1.3          |
| Vitamin–mineral premix | 3.5         | 3.5          |
| Lysine               | 1.6           | 1.6          |
| **Chemical composition** |             |              |
| ME (kcal/kg)         | 3300          | 3030         |
| Dry matter (%)       | 89.40         | 90.50        |
| CP (%)               | 23.62         | 19.25        |
| Ether extract (%)    | 6.1           | 8.9          |
| Crude ash (%)        | 5.1           | 4.3          |
| Crude fibre (%)      | 5.6           | 5.0          |
| Calcium              | -             | 10.2         |
| Total phosphorus     | 5.5           | 5.6          |

**Notes:**

- Vitamin and mineral premix provided per kilogram of diet: retinol, 4.5 mg; cholecalciferol, 0.125 mg; alpha-tocopherol, 100 mg; menadione, 4 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 60 mg; pyridoxine, 5 mg; Ca-o-pantothenate, 18 mg; folic acid, 2 mg; b-biotin, 0.20 mg; Mn, 100 mg; Zn, 80 mg; Fe, 80 mg; Cu, 8 mg; Co, 8 mg; Se, 0.3 mg; iodine, 1 mg, Mo, 1 mg; choline chloride, 500 mg.
- Analysed (AOAC 1995).
- Based on NRC (1994) feed composition tables.
3. Results and discussion

The fatty acids composition of starter, finishing diets and EEP is presented in Tables 3 and 4. The main fatty acids contained in starter and finishing diets are linoleic acid (around 39%, 47%, respectively) and oleic acid (around 28%, 18%, respectively). The fatty acids composition of lipids from quail internal organs is presented in Table 5. The different levels of propolis supplementation enhanced PUFA levels of internal organs. Increased PUFA levels of kidney (P < .01) and liver (P < .01) tissues are measured in propolis-supplemented groups in comparison with the HS group. This finding might be due to the antioxidative capacity of propolis. The antioxidative capacity of propolis might have a positive effect on fatty acids composition of the internal organs of quail feeding under stocking density. Morrissy et al. (1994) reported that lipid peroxidation caused a reduction of PUFA in phospholipid fraction of the tissues. Our present results indicate that decreased levels of PUFA of tissue in unsupplemented HS group compared to supplemented HS groups. This finding might be linked to the use of unsaturated fatty acids as an energy source for lipid metabolism under stress conditions. Propolis supplementation decreased the stress induced by high stocking density. Significantly higher levels of saturated fatty acids (SFA) are found in the kidney (P < .05) and liver (P < .05) of the HS group than those of other groups, whereas those of abdominal fat are similar in all groups. This finding might be due to high or increased stocking density. In the previous study, Tatlı Seven et al. (2009) reported that the antioxidative-effective propolis showed effects by decreasing lipid peroxidation caused by stress. The PUFA levels of tissues of HS-0.5, HS-1 and HS-1.5 groups are significantly higher than those of the HS group. This finding is consistent with our previous research (Seven et al., 2009).

Table 2. Chemical composition assessed by GC-MS of propolis.a

| RT (min) | Contents                                      | TIC (%) |
|---------|-----------------------------------------------|---------|
| 4.787   | Benzyl alcohol                                 | 0.279   |
| 11.334  | 2-Methoxy-4-vinylphenol                        | 0.550   |
| 11.047  | Cinnamal alcohol                               | 0.455   |
| 8.309   | Benzoic acid                                   | 1.123   |
| 24.097  | Isoferulic acid                                 | 0.908   |
| 28.866  | p-Coumaric acid                                | 0.404   |
| 14.418  | Cinnamic acid                                  | 1.043   |

Table 3. The fatty acids composition of diets (g/kg).

| C Number | Name                                      | Starter | Finishing |
|----------|-------------------------------------------|---------|-----------|
| C14:0    | Myristic acid (tetradecanoic acid)         | 2.4     | 2.4       |
| C15:0    | Pentadecanoic acid (pentadecanoic acid)   | 0.5     |           |
| C16:0    | Palmitic acid (hexadecanoic acid)          | 161.2   | 174.6     |
| C17:0    | Margaric acid (heptadecanoic acid)         | 2       | 2.2       |
| C18:0    | Stearic acid (octadecanoic acid)           | 54.1    | 26.8      |
| C20:0    | Arachidic acid (eicosanoic acid)           | 4.2     | 5.1       |
| C22:0    | Behinic acid (dokosanoic acid)             | 1.9     | 2.8       |
| C24:0    | Lignoceric acid (docosanoic acid)          | 1.4     |           |

Notes: RT: Retention time (minute). TIC: Total ion current.

Table 4. The fatty acids composition of ethanolic extract of propolis (EEP) (%).

| C Number | Name                                      | %       |
|----------|-------------------------------------------|---------|
| C10:0    | Capric acid (decanoic acid)               | 2.0     |
| C11:0    | Undecenoic acid (undecenoic acid)         | 0.47    |
| C12:0    | Lauric acid (dodecanoic acid)             | 3.43    |
| C14:0    | Myristic acid (tetradecanoic acid)         | 2.19    |
| C15:0    | Pentadecanoic acid (pentadecanoic acid)   | 0.10    |
| C16:0    | Palmitic acid (hexadecanoic acid)          | 11.26   |
| C17:0    | Margaric acid (heptadecanoic acid)         | 1.29    |
| C18:0    | Stearic acid (octadecanoic acid)           | 3.44    |
| C18:1    | Oleic acid (eicosanoic acid)               | 5.67    |
| C18:2    | Linoleic acid (dokosanoic acid)            | 24.82   |
| C18:3    | Omega-3 fatty acids (cis-9,12,15-entatrienoic acid) | 30.6 |
| C20:2    | Omega-2 fatty acids (cis-11,14-eicosadienoic acid) | 6.0 |
| C20:4    | Omega-4 fatty acids (cis-8,11,14,17-eicosatetraenoic acid) | 0.4 |
| C20:5    | Omega-3 fatty acids (cis-5,8,11,14,17-eicosapentaenoic acid) | 0.6 |
| C22:2    | Docosadienoic acid                         | 0.5     |

Table 5. The fatty acids composition of starter, finishing, and ethanolic extract of propolis (EEP) (%).

| C Number | Name                                      | %       |
|----------|-------------------------------------------|---------|
| C14:0    | Myristic acid (tetradecanoic acid)         | 2.4     |
| C15:0    | Pentadecanoic acid (pentadecanoic acid)   | 0.5     |
| C16:0    | Palmitic acid (hexadecanoic acid)          | 161.2   |
| C17:0    | Margaric acid (heptadecanoic acid)         | 2       |
| C18:0    | Stearic acid (octadecanoic acid)           | 54.1    |
| C20:0    | Arachidic acid (eicosanoic acid)           | 4.2     |
| C22:0    | Behinic acid (dokosanoic acid)             | 1.9     |
| C24:0    | Lignoceric acid (docosanoic acid)          | 1.4     |

Notes: RT: Retention time (minute). TIC: Total ion current.

*The ion current generated depends on the characteristic of the compound concerned and it is not a true quantification.
higher than those of the HS group. It can be attributed to the prevention of use of PUFA by propolis supplementation.

Generally, customers prefer poultry meat due to the low fat content and high PUFA content. In a previous study Simsek et al. (2009) reported that a lower stocking density decreased the fat ratio of meat and increased the protein ratio, total PUFA and n-6 fatty acid ratio. Our present study indicated that the different levels of propolis additives enhanced PUFA (P < .01) and n-6 PUFA levels. n-6 PUFA levels of kidney (P < .05) tissue are found higher in propolis-supplemented groups than those of HS group.

These findings are in accordance with those reported by Simsek et al. (2009). The effects of supplementation of Propolis on serum MDA, some biochemical parameters and fatty acids composition of internal organ tissues were determined. Serum MDA levels of HS, HS-0.5, HS-1 and HS-1.5 groups are found as 6.84, 4.33, 4.08 and 4.56 nM/mL, respectively (Table 6). It is obvious that serum MDA activity is decreased by propolis supplementation in quail reared under stocking density. Serum MDA levels of the HS group are found to be significantly (P < .01) higher than those of HS-0.5, HS-1 and HS-1.5 groups. Stocking density may enhance oxidative destruction (Celik et al. 2010) and cause MDA generation (Simsek et al. 2009). The effects of supplementation of Propolis along with paclitaxel on dimethyl benzanthracene-induced experimental breast cancer in female rats. They concluded that propolis is a potent antioxidant and the administration of propolis at a dose of 50 mg/kg bw with paclitaxel decreases the toxic complications of chemotherapy and increases the levels of free radical scavenging enzymes. Our present results showed that stocking density caused oxidative stress and enhanced serum MDA levels. The supplementation of propolis in diet decreased the serum MDA levels. It could be concluded that the rich flavonoid content of propolis might be effective.

The levels of serum albumin (P < .05), creatinine (P < .05), globulin (P < .001), total protein (P < .001), ALT (except for HS-5

Table 5. The fatty acids composition of kidney, liver and abdominal fat.

| Fatty acids | HS   | HS-0.5 | HS-1  | HS-1.5 | P    |
|------------|------|--------|-------|--------|------|
| Kidney     |      |        |       |        |      |
| PUFA       | 365.8 ± 31.7b | 445.0 ± 8.1a | 445.4 ± 5.2a | 439.1 ± 2.6a | **  |
| MUFA       | 192.3 ± 21.2 | 168.5 ± 7.1 | 182.1 ± 21.9 | 181.5 ± 5.1 | NS  |
| SFA        | 437.1 ± 32.2b | 381.7 ± 9.6b | 365.4 ± 16.4b | 373.5 ± 7.1b | *   |
| n-6PUFA    | 342.3 ± 33.9b | 417.2 ± 9.6a | 421.2 ± 7.2a | 419.1 ± 5.1a | *   |
| Liver      |      |        |       |        |      |
| PUFA       | 377.6 ± 36.1b | 415.5 ± 13.5b | 423.2 ± 29.3b | 438.5 ± 26.2ab | **  |
| MUFA       | 248 ± 22.4 | 244.1 ± 23.4 | 221.1 ± 22.3 | 228.5 ± 34.3 | NS  |
| SFA        | 369.8 ± 17.5b | 340.4 ± 10.7b | 321.9 ± 17.6b | 332.0 ± 6.9ab | *   |
| n-6PUFA    | 332.1 ± 31.1b | 366.7 ± 30.6b | 394.2 ± 22.3b | 373.7 ± 20.9ab | *   |
| Abdominal fat |      |        |       |        |      |
| PUFA       | 203.2 ± 8.2 | 232.0 ± 15.6 | 233.8 ± 22.1 | 240.9 ± 6.9 | NS  |
| MUFA       | 355.4 ± 22.2 | 343.6 ± 15.4 | 352.9 ± 12.3 | 350.4 ± 5.1 | NS  |
| SFA        | 439.5 ± 11.8 | 420.5 ± 5.8 | 404.3 ± 18.5 | 401.2 ± 10.1 | NS  |
| n-6PUFA    | 177.6 ± 16.3 | 200.6 ± 14.8 | 207.9 ± 18.8 | 212.4 ± 13.5 | NS  |

Notes: All values were presented as means ± SE; NS: Non-significant *P < .05, **P < .01, ***P < .001. Mean values with different superscripts within a row differ significantly. HS: High stocking and basal diet; HS-0.5: High stocking and basal diet supplemented with 0.5 g propolis/kg; HS-1: High Stocking and basal diet supplemented with 1 g propolis/kg; HS-1.5: High stocking and basal diet supplemented with 1.5 g propolis/kg.

The levels of serum MDA and some biochemical parameters of experimental groups.

| Parameter          | HS     | HS-0.5 | HS-1 | HS-1.5 | P    |
|--------------------|--------|--------|------|--------|------|
| Serum MDA (nM/mL)  | 6.84 ± 0.28bc | 4.33 ± 0.33bc | 4.08 ± 0.24bc | 4.56 ± 0.40bc | **  |
| Glucose (mg/dL)    | 333.60 ± 7.15 | 338.00 ± 12.35 | 318.66 ± 7.66 | 324.62 ± 11.90 | NS  |
| Albumin (g/dL)     | 1.20 ± 0.05a | 1.00 ± 0.06bc | 1.01 ± 0.05b | 1.02 ± 0.03b | *   |
| Creatinin (mg/dL)  | 0.30 ± 0.05a | 0.20 ± 0.01b | 0.21 ± 0.01b | 0.20 ± 0.01a | *   |
| Globulin (g/dL)    | 2.07 ± 0.05a | 1.52 ± 0.04b | 1.46 ± 0.06b | 1.42 ± 0.08b | *** |
| Total protein (g/dL)| 3.22 ± 0.10bc | 2.51 ± 0.06bc | 2.43 ± 0.12bc | 2.44 ± 0.13bc | *** |
| Urea (mg/dL)       | 3.00 ± 0.31 | 2.66 ± 0.33 | 2.83 ± 0.30 | 2.62 ± 0.26 | NS  |
| ALT (U/L)          | 3.33 ± 0.88b | 2.83 ± 0.30ab | 2.00 ± 0.31b | 2.12 ± 0.22b | **  |
| AST (U/L)          | 215.80 ± 2.37 | 193.00 ± 7.97 | 192.00 ± 12.88 | 198.12 ± 15.79 | NS  |
| LDL cholesterol (mg/dL) | 102.60 ± 1.50 | 88.83 ± 4.71b | 83.16 ± 2.61b | 82.00 ± 1.56c | *** |

Notes: All values were presented as means ± SE; NS: Non-significant *P < .05, **P < .01, ***P < .001. Mean values with different superscripts within a row differ significantly. HS: High stocking and basal diet; HS-0.5: High stocking and basal diet supplemented with 0.5 g propolis/kg; HS-1: High Stocking and basal diet supplemented with 1 g propolis/kg; HS-1.5: High stocking and basal diet supplemented with 1.5 g propolis/kg.
group ($P < .01$) and LDL cholesterol ($P < .001$) in the HS group are significantly higher than those of other groups (Table 6). Our present results indicate that the levels of glucose, urea and AST in blood were not influenced by propolis treatments. These results are in accordance with those of previous authors (Biavatti et al. 2003; Denli et al. 2005).

Stocking density is a stress factor for poultry (Celik et al. 2010). Corticosterone secreted by the adrenal gland in response to stressors increases blood through gluconeogenesis (Tiftik 1996). AST levels of blood increase with stress. The activities of AST and ALT enzymes increase with hepatic damage (Tiftik 1996). While ALT levels of HS group are significantly higher than those of HS-1.0 and HS-1.5 group, AST levels are not found significant in this study. Another study (Denli et al. 2005) reported that the supplementation of propolis in diet caused a reduction in AST and ALT activities. According to these findings, Denli et al. (2005) concluded that propolis might have hepatoprotective effects or played a role in the prevention of liver injury.

4. Conclusions

Stocking density caused stress and enhanced MDA levels. The different levels of propolis supplementation decreased negative effects of stocking density. Propolis had positive effects on antioxidant metabolism and decreased the serum MDA level. So, it can be used as an additive in the ration of poultry because of antioxidant capacity and potent protective activity on fatty acid composition of quail tissue under high stocking density stress.

Disclosure statement

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