The effects of ozonated, chlorinated, celestite stone-treated, natural spring and pine resin-treated waters on performance, oxidative stress and carcass parameters in Japanese quail

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ABSTRACT
This study was conducted to assess the effects of 5 types of water: Ozonated, Chlorinated, Celestite Stone-treated, Natural Spring Waters and Pin Resin-treated water on performance, oxidative stress and carcass parameters in Japanese quail. A total of 150, 3-day old mixed female-male quails were used into 5 groups of 30 birds for each water group; each group was divided into 3 replicate subgroups of 10 birds. The experiment was continued for 7 weeks and quail were fed one experimental diet, and the different water sources were given ad libitum during in the trial. Statistical differences among water sources were observed on pH and Electrical Conductivity and content of Ca, Mg, Cl and Total Bacteria. Live weight, daily live weight gain, water and feed intake were the highest in the drinkers of Natural Spring Water, while the lowest for drinkers of Resin-treated Water (p<0.001). The lowest level of serum malondialdehyde of was observed in drinkers of Natural Spring Water (1.54 µM/L), and the highest malondialdehyde level in drinkers of Resin-treated Water (4.27 µM/L; P<0.001). The sources of water of trial were determined to have no effects on slaughter weight, carcass weight and carcass yield (p>0.05). As a result, the amount of total dissolved solids of water were the positive effect on live weight, feed consumption, water intake, feed conversion ratio and oxidative stress biomarkers.

Keywords: Celestite stone-treated water, chlorinated water, ozonated water, resin-treated water, water types

Introduction
Water is a vital for the life. The water content of poultry is 55-77% of total body weight although it varies according to species, age and sex; intracellular fluid, contained within cells, accounts for approximately 2/3 of body water and extracellular fluid accounts for 1/3 of body water (Swenson and Reece, 1993). Water consumption of poultry is 83 % (70-97 %) provided by directly from the drinking water, and the balance obtained by oxidative metabolism and water consumed in feeds. Water intake is equal to the amount of water lost in feces, urine and breathing (Cemek et al., 2011; Eleroglu et al. 2013). Lott et al. (2003) reported a correlation of 98% between water consumption and feed consumption. Although water quality of poultry or quail has been researched or reviewed in Turkey (Cemek et al., 2011; Eleroglu et al. 2013; Ozdogan et al. 2016) and the world (Marks, 1981; Minvielle et al., 2007; Ragab, 2008; Nain et al., 2011), and we could not find a study on poultry or quail on the effect of the sources of water similar to those used in our experiment.

Materials and Methods
Bird Management and Diet: In the study, a total of 150 Japanese quail (Coturnix coturnix japonica) were used as experimental animals. Day-old chicks were supplied from a special quail farmer in Elazig and placed on experiment from the age of 3 days. This study was started after being approved by Bingol University Animal Experiments Local Ethic Committee (Date: 11.07.2014, Decision No: 2014-03), and carried out at Department of Zootechnic Poultry Breeding Unit at
Bingol University. Mixed female-male quail were randomly assigned to 5 groups of 30, and divided into 3 replicate subgroups of 10 birds each. The experiment was run for 7 weeks. Ten chicks were housed in plastic cages of width 34 cm, height 62.5 cm and depth 43 cm with a cleanable base and 5 liter water reservoirs. During the experiment a single diet (Crude Protein, CP 26.14% and Metabolic Energy, ME 3069 kcal/kg) was fed ad libitum to meet all nutrient requirements of quail according to National Research Council (NRC, 1994), (Table 1). Five different waters were given ad libitum for the quail. Sources of waters used in the experiment are given in Table 2. In the study, water intake was calculated weekly by dividing water consumption by number of animals and days. Animals were exposed to light for 24 hours/day during the experiment.

**Analyses of Waters, Feed, Bloods and Carcass:**
Waters were analyzed for pH, Na, K, Cl, Ca, Mg, Electrical Conductivity (EC), total bacteria and fecal bacteria (E. coli) according to Nollet and Gelder (2014) in the Soil Department Laboratory and Central Laboratory of Bingol University. EC is also a Total Dissolved Solids (TDS) indicator in water (FAO, 2016, Verma 1995). Samples of feeds of the experiment were analyzed according to Association of Official Analytical Chemists (AOAC 1990) for dry matter by oven-drying and for ash (Nuve Laboratory Equipment, Istanbul, TR), ether extract (Velp Scientifics, Milan, IT) and crude protein (Dumatherm, DE) in Central of University of Bingol Laboratory. Metabolic energy of diets was calculated according to Pauzenga (1985). Feed conversion rate (FCR) were determined by daily feed intake/average daily gain (ADG). At the end of the experiment, 4 male and 4 female quails were selected at random from each group and venous bloods were taken into 10 ml tubes with EDTA. Supernatants obtained by centrifugation at 3000 rpm for 10 min. were stored at -80 °C until analysis. The oxidative stress biomarkers of the serums were measured in a private laboratory (Oksante Lab., Istanbul) via a cold chain. Serum malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase (CAT) enzyme activities were determined according to Buege and Aust (1978). Ewing and Jenaro (1995) and Goth (1991). At 52 days of age of quail or on slaughter day, weights of the organs of the digestive tract except the liver and kidney were separated from those bodies of 5 male and 5 female animals from each group and “hot carcass” weights determined; hot carcass weights were divided by the slaughter weight and “carcass yield” calculated according to Konca et al. (2015).

### Table 1. Composition of experimental diet

| Ingredients | % | Analyzed | % |
|-------------|---|----------|---|
| Corn        | 50| DM       | 89.58 |
| Soybean meal, 48% CP | 40| Ash | 7.03 |
| Canola Oil  | 3.0| Crude Protein | 26.14 |
| Di Calcium Phosphate | 2.25| Crude Cellulose | 4.05 |
| Limestone, 38% Ca | 3.9| Ether Extract | 6.41 |
| Vitamin Premix 1 | 0.15| NFE 3 | 45.95 |
| Trace Element Premix 2 | 0.1| AME, kcal/kg 4 | 3095 |
| Ethoxyquin   | 0.1|          |     |
| DL-Methionine| 0.2|          |     |
| L-Lysine HCL | 0.05|          |     |
| Salt         | 0.25|          |     |

1: DSM Rovimix 124® per kg: 6500 000 IU Vitamin A, 1500 000 IU Vitamin D3, 25 000 mg Vitamin E, 2500 mg Vitamin K3, 1500 mg Vitamin B1, 3000 mg Vitamin B2, 2500 mg Vitamin B6, 15 mg Vitamin B12, 25000 mg Vitamin C, 5000 mg Calcium D-Pantothenate, 15000 mg Niacin, 500 mg Folic acid, 30 mg Biotin, 250 mg Apo carotenoid acid ester, 62500 mg Endox D Dry. 2: DSM Remineral S® in each kg: 800000 IU Vitamin A, 150000 IU Vitamin D3, 5000 mg Mn, 6000 mg Fe, 6000 mg Zn, 5000 mg Co, 200 mg Cu, 1000 mg I, 150 mg Se, 300000 mg choline chloride. 3: NFE, Nitrogen Free Extract, = % DM- (Crude Protein, % + Crude Cellulose, % + Crude Fat, % + Ash, %). 4: AME, Apparent Metabolic Energy, kcal / kg = 37 x Crude Protein, + 81 x Crude Fat, + 35 x NFE, % (Pauzena 1985).

### Table 2. Characteristics of sources of water in the experiment

| Sources of Water | Characteristics |
|------------------|-----------------|
| Ozonated Water   | Commercial ozonated bottled water produced in Solhan District of Bingol was used. |
| Chlorinated Water| Water of city network in Bingol was used. |
| Celestite Stone-treated Water | During the trial, 200 g of celestite stone was placed in each reservoir, and 4.5 liters of city water was added. Celestite, strontium sulfate (SrSO4) is considered a stone that reduces stress by the people in Turkey. |
| Natural Spring Water | Natural spring water flowing from Karlova Fountain near Bingol Central Women’s and Children’s Hospital was used. |
| Resin-treated Water | During the trial, 100 g of resin pine wood pieces was placed in each reservoir, and 4.5 liters of city water was added. |
Statistical Analysis: A one-way ANOVA analysis was used to assess the significance of the differences between the mean values of the water groups and significant differences of means were determined by Duncan Test of SPSS (1993).

Results

Effects of waters in the experiment were given at Tables 3-8. The results of the analyses of five different water sources used in the experiment are given in Table 3. The highest 7.49 of pH value of types of water was in Natural Spring Water (p<0.018) and the pH ranking of waters were Ozonated, Celestite Stone-treated, Chlorinated Waters and Resin-treated Water. The effects of the waters on live weight and ADG of quails are presented in Tables 4-5. The live weight (except 2nd week) and ADG (except 4-7 days) of the group drinking Natural Spring Water was higher than the other group (p<0.001). The effects of waters of sources on feed consumption (p<0.001) and FCR (except 1-3 weeks) (P<0.002) in the other waters in the experiment. The reasons for the high pH of Natural Spring Water is that high values of Mg, Na, Ca and K or increasing Total Dissolved Solid (TDS) are found in this water. The lowest pH value was found in Resin-treated Water, which was produced by passing water through resins of pine pieces, resulting in acidification of the water. There were no statistically significant differences in Na and K concentrations of the waters (p>0.05) and E coli

Table 3. Results of analysis of some parameters of water sources in the experiment

| Parameters | Sources of Waters                      |  |  |  |  |  |  |  |  |
|------------|---------------------------------------|---|---|---|---|---|---|---|---|
|            | Ozonated Water                        | Chlorinated Water | Celestite Stone-treated Water | Natural Spring Water | Resin-Treated Water | Water Standards* | p   |
| pH         | 7.00 b                                 | 6.89 bc            | 6.94 b                        | 7.49 a              | 6.30 c             | 6.5-9.5          | 0.018 |
| Ca (ppm)   | 7.47 c                                 | 10.77 bc           | 11.93 b                       | 11.90 b             | 13.13 a            | 5-50             | 0.029 |
| Mg (ppm)   | 18 c                                   | 20 bc              | 22 b                          | 24 a                | 23 a               | 10-50            | 0.035 |
| Na (ppm)   | 2.30                                   | 2.27               | 2.47                          | 3.00                | 2.30               | 20-175           | 0.27  |
| K (ppm)    | 1.70                                   | 2.20               | 2.90                          | 1.97                | 2.87               | 12               | 0.12  |
| Cl (ppm)   | 0.10 b                                 | 0.34 a             | 0.20 ab                       | 0.05 c              | 0.26 a             | 5                | 0.005 |
| E.C (Micro s/cm) | 61.17 d                             | 89.33 c            | 171.33 b                      | 212.00 a            | 110.07 c           | 2500             | 0.001 |
| T.B. (CFU/ml) | 286 c                              | 370 b              | 391 b                         | 566 a               | 253 c              | 1000             | 0.038 |
| E.coli (CFU/ml) | 0                                  | 0                  | 0                             | 0                   | 0                  | -                |       |

E.C: Electrical conductivity, T.B: Total bacteria, a, b, c, d: The significant differences between the averages are shown by different letters on the same line, *: Cemek et al. 2011; Tabler et al., 2015; FAO, 2016.

Table 4. Effects of water sources on the average live weight of Japanese quail, (g/bird).

| Sources of Water | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|------------------|-------|-------|-------|-------|-------|-------|-------|
| Ozonated Water   | 21.67 bc | 59.43 a | 90.70 b | 136.80 b | 157.00 b | 171.68 b | 190.97 b |
| Chlorinated Water| 22.13 bc | 50.76 c | 86.20 bc | 130.50 c | 153.17 b | 167.67 bc | 187.03 b |
| Celestite Stone-treated Water | 20.26 c | 57.03 ab | 89.36 bc | 135.73 b | 153.90 b | 173.18 bc | 190.20 b |
| Natural Spring Water | 23.46 a | 59.20 ab | 99.13 a | 147.46 a | 166.37 a | 182.90 a | 202.17 a |
| Resin-treated Water | 19.43 c | 49.93 c | 85.80 c | 126.73 d | 147.03 c | 163.23 c | 175.17 c |

P values
- 0.050
- 0.001
- 0.001
- 0.001
- 0.001
- 0.001
- 0.001

a, b, c; The significant differences between the averages are shown by different letters on the same column.

Table 6. Daily water intake/daily feed intake in Figure 1 and daily water intake/live weight in Figure 2. Consumption of Natural Spring and Resin-treated waters by quails were found to be statistically higher than the other 3 groups during in the experiment (p<0.001). The effects of water sources on MDA, SOD and CAT in serum are presented in Table 7. In general, average serum MDA level was found to be lower (p<0.001) in the Natural Spring water group than other groups especially Resin-treated Water group. The effects of water type on slaughter weight, hot carcass weight and carcass yield are presented in Table 8. No statistical differences were found among groups (p>0.05).

Discussion and Conclusion

The reason for the high pH of Natural Spring Water is that high values of Mg, Na, Ca and K or increasing Total Dissolved Solid (TDS) are found in this water. The lowest pH value was found in Resin-treated Water, which was produced by passing water through resins of pine pieces, resulting in acidification of the water. There were no statistically significant differences in Na and K concentrations of the waters (p>0.05) and E coli

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undetectable in all waters. Ca and Mg concentrations are excessive in the Resin-treated water because of retention of cations in the acidic medium (Verma 1995). The results of all water analysis showed that the waters in the experiment are appropriate according to standards of poultry water (Cemek et al. 2011; Tabler et., 2015; FAO, 2016).

Increasing live weight and ADG of the Natural Spring Water group may be caused by high of concentrations of Mg and Na or increasing of TDS in the water. Live weight and ADG of the Resin-treated Water group of quail were significantly lower than other groups. We think that the reasons for this decrease, stemmed from acidification of the water, decreased feed intake, the watery feces and excessive water intake. Eleroglu et al. (2013) reported that the hardness, pH and dissolved oxygen of drinking water had positive effects on live weight and ADG. Marks (1981) reported that live weight and ADG of quail depend on the protein content in diet and 20% and 28% of protein of diets resulted in, respectively, live weights of 30.1 and 41.5 g at day 14.

Kilany and Mahmoud (2014) determined that a diet with 0.5% of Turmeric (Curcuma longa L.) was between 6.06-6.43 g/day on ADG for 7 weeks. Kaplan et al. (2005) reported that 1% NaHCO₃ in diet gave ADG between 2.70-4.73 g for mixed-sex quail in 7 weeks under temperature stress. Decreased feed consumption and FCR of Resin-treated Water group appears to be caused by acidification of the water and excessive water consumption. The Ozonated, Chlorinated and Celestite Stone-treated waters were similar to each other in their effects on feed intake and FCR values during in the trial. Marks (1981) reported that intakes on 20 and 28% dietary protein were, respectively, 12.2 and 15.7 g/day/bird and feed consumption increased with increasing protein level in diet. In our study, feed consumption and FCR values were similar to results of Konca et al. (2015), Kilany and Mahmoud (2014) and Macleod and Dabutha (1997).

Despite the increased water intake, feed consumption, live weight, ADG and FCR were all

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**Table 5. Effects of the water sources on average daily gain (ADG), feed intake and feed conversion rate (FCR) of Japanese quail.**

| Sources of Water       | ADG (g/day/bird) | Feed Intake (g/day/bird) | FCR (g/g) |
|------------------------|------------------|--------------------------|-----------|
|                        | 1-3 wk | 4-7 wk | 1-7 wk | 1-3 wk | 4-7 wk | 1-7 wk | 1-3 wk | 4-7 wk | 1-7 wk |
| Ozonated Water         | 4.32a  | 3.58a  | 3.90b  | 11.30b | 22.51b | 17.70b | 2.62    | 6.30b  | 4.54b  |
| Chlorinated Water      | 4.10b  | 3.60a  | 3.82b  | 11.03b | 22.17b | 17.40b | 2.69    | 6.16b  | 4.56b  |
| Celestite Stone-treated Wt. | 4.25b  | 3.60a  | 3.88b  | 11.70b | 22.55b | 17.90b | 2.75    | 6.27b  | 4.61b  |
| Natural Spring Water   | 4.72a  | 3.68a  | 4.13a  | 13.33a | 25.51a | 20.29a | 2.82    | 6.93a  | 4.92a  |
| Resin-treated Water    | 4.08c  | 3.19b  | 3.57c  | 10.17c | 19.48c | 15.49c | 2.49    | 6.12b  | 4.33c  |
| P values               | 0.001  | 0.025  | 0.001  | 0.001  | 0.001  | 0.001  | 0.081   | 0.020  | 0.002  |

**Table 6. Effects of water sources on average daily water intake of Japanese quail, g/day/bird**

| Sources of Water       | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 1-3   | 4-7   | 1-7   |
|------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Ozonated Water         | 11.00a| 26.43b| 34.76c| 39.50b| 44.53c| 53.23b| 57.73c| 24.06c| 48.76c| 38.16c|
| Chlorinated Water      | 12.26b| 26.26b| 34.73b| 38.56b| 43.26c| 52.06b| 55.26c| 24.43c| 47.30c| 37.50c|
| Celestite Stone-treated Wt. | 11.73c| 26.43b| 34.46c| 39.36b| 43.83c| 54.43c| 58.23c| 24.26c| 48.96c| 38.36c|
| Natural Spring Water   | 13.03c| 29.46a| 37.53c| 45.10a| 53.60a| 59.36c| 68.33a| 26.66b| 56.60a| 43.76a|
| Resin-treated Water    | 13.40c| 31.13a| 40.16a| 43.30a| 48.60b| 58.30c| 64.96b| 28.23c| 53.83b| 42.83b|
| P values               | 0.005 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

**Note:** The significant differences between the averages are shown by different letters on the same column.
reduced in quail given Resin-treated Pine or other resin particles are used for disinfection and softening of waters in rural and forest areas in Turkey. The group given Acidic Resin-treated Water was the highest among the groups. The Daily Water Consumption/Live Weight rates are given in Graph 2. While live weights increased, the rates vertically decreased until the 4th weeks. This decrease was also confirmed by Shim and Vohra (1984). Nain et al. (2011) reported that the average daily consumption of water was 29.8 ml/bird on a 12:12 light: darkness program at 3 weeks of age. The water consumption of the female quail at 7 weeks of age was 55.37 g/day, and the daily water consumption/live weight ratio was 1.66 g/g determined by Minvielle et al. (2007). In our experiment, the daily consumptions of water in all groups were similar to Marks (1981), Nain et al. (2011) and Minvielle et al. (2007).

The reason for the increased serum MDA of the Resin-treated Water group may have been because of decreased oxygen metabolism in cells because of acidic water. In addition, that group had increased water intake, decreased feed consumption and FCR. Wang et al. (2016) reported that the effects of adding 3 g/kg of Arctium lappa L. root in the diet reduced serum MDA to 7.51 nmol/ml compared to 9.21 nmol/ml in the control group, and also reduced serum SOD values to 215.72 U/ml versus 234.73 U/ml in the control. Hsu et al. (2015) investigated the effects of 18 mg/kg of bacterial lycopene on oxidative stress in 100-day old laying quail were 52.3 μM of serum MDA in control group and 24.5 μM of the lycopene group and SOD values were 46.1 U/ml and 59.4 U/ml, respectively. Kilany and Mahmoud (2014) found that 0.5% Turmeric (Curcuma longa L.) of diet were 0.68 μg/ml of the serum MDA, 160 U/ml of SOD and 58.75 U/ml of CAT in the quails. Researchers have reported that Turmeric reduced oxidative stress in quail, while serum MDA was lower than control group. Likewise SOD and CAT values were found to be higher than control group.

We found no studies in the literature that examined effects of water type on these parameters. However, it...
can be said that these values are consistent with the values of carcass weight and carcass yield which are reported by Konca et al (2015), Oguz et al. (2011) and Yildirim and Ozturk (2013).

We have reached the following conclusions in the study: resin treatment did not make a significant contribution to disinfection and softening of the water. TDS appears to affect live weight, feed consumption and water content. Water varieties also affect oxidative stress in different ways. Results of the study indicate that, except for Resin-treated Water, supplying Natural Spring, Ozonated, Celestite Stone Waters or Chlorinated Water to growing Japanese quail yields satisfactory animal performance.

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