



The Effect of Thyme (*Thymbra spicata L. var. spicata*) Essential Oil on the Antioxidant Potential and Meat Quality of Japanese Quail Fed in Various Stocking Densities *

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Abstract: This study was conducted to determine the antioxidant effect of the Thyme (*Thymbra spicata L. var. spicata*) essential oil on meat quality of Japanese Quail fed in various stocking densities. In this study, a total of up to 7-day age 300 Japanese Quails (*Coturnix coturnix Japonica*) chickens were used and the experimental period lasted for 28 days. The chickens were divided into 6 groups, each containing 50 chicks Control group (CONT), High stocking density control group (HSD-CONT), an antibiotic group (HSD-ANT), zahter oil groups (HSD-T₁, T₂, T₃). At the end of the study, serum MDA level was found significantly higher in the HSD-ANT group than the other groups (P<0.01). It was determined that the stocking density has a significant effect on the pH and colour of brisket (P<0.01). Moreover, inclusions of thyme essential oil supplementation into the diets of quails fed in high stocking density provided various levels of improvements on antioxidant potential, and especially thyme EO at 600 mg per kg were found more effective to ameliorate the detrimental effects of oxidative stress caused by high stocking density.

Keywords: Antioxidant, Japanese Quail, Stocking Density, Thyme Essential Oil.

Zahter (*Thymbra Spicata L. var. Spicata*) Uçucu Yağının Farklı Yerleşim Sıklığında Beslenen Japon Bildircinlerinde Antioksidan Potansiyel ve Et Kalite Parametrelerine Etkisi

Öz: Bu çalışma zahter (*Thymbra spicata L. var. spicata*) uçucu yağının farklı yerleşim sıklığında beslenen Japon bildircinlerinde antioksidan potansiyelini ve et kalitesine etkisini belirlemek amacıyla yapılmıştır. Çalışmada, 7 günlük yaşta, toplam 300 adet Japon bildircin (*Coturnix coturnix japonica*) civciv kullanılmış ve çalışma 28 gün sürdürülmüştür. Civcivler, her biri 5 tekerrür ve 50 civcivden oluşan 6 gruba ayrılmıştır. Araştırma grupları; normal yerleşim sıklığında kontrol grubu (NYS-KONTROL), yoğun yerleşim sıklığında kontrol grubu (YYS-KONT), yoğun yerleşim sıklığı uygulanan gruplara sırayla 200, 400, ve 600 mg/kg zahter uçucu yağı ve 10 mg/kg *Avilamin* katkısı yapılan grup (YYS-ANT) şeklinde dizayn edilmiştir. Araştırmada, 50x100 cm ebadındaki kafeslerde, normal yerleşim sıklığı 160 cm²/bildircin; yoğun yerleşim sıklığı ise 90 cm²/bildircin olarak düzenlenmiştir. Çalışma sonunda, serum MDA düzeyinin YYS-ANT grubunda diğer gruplara göre önemli derecede yüksek olduğu belirlenmiştir (P<0.01). Araştırma sonunda, en düşük göğüs eti pH'sı YYS-KONT grubunda belirlenirken üzerine yerleşim sıklığının etkisi önemli (P<0.01) bulunmuştur. Sonuç olarak, yerleşim sıklığı uygulanan bildircin rasyonlarına zahter uçucu yağı ilavesi, lipid peroksidasyonu üzerinde doza bağlı olarak iyileşmeler sağlamış ve özellikle 600 mg/kg zahter uçucu yağının daha etkili olduğu belirlenmiştir.

Anahtar Kelimeler: Antioksidan, Bildircin, Performans, Uçucu Yağ, Yerleşim Sıklığı.

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INTRODUCTION

Antioxidants are the substances which increase the product quality by inhibiting the oxidation of substrate or delaying the oxidation at very low concentrations in feeds and meat. Antioxidants can be used as food additives or supplements to stabilize the feeds and prevent the component loss in the feeds. The mechanism of feed preserving antioxidants are to eliminate the metal ions or oxygen activities or to prevent the attachment of the free radicals by sending an electron or hydrogen atom (1,2).

In poultry farming, the oxidative stressors such as density, high or low temperature, transportation and vaccination directly affect the quality parameters of meat. Stressors cause the stored glycogen to transform into glucose. In case of prolonged stress conditions the glucose transforms into lactic acid resulting in dark coloured, dry, undesirable and inadequately marbled with high pH and short shelf life meat (3). Post slaughter meat quality is primarily affected by the transformation of the glycogen stored in muscles into lactic acid and the water retention capacity and colour are affected directly depending on the hydrolysis features of the meat proteins (4). In the post-slaughtering period when carcass temperature is 37 °C, a rapid decline in pH causes to shrink myofibril proteins, deteriorate sarcoplasmic proteins and make the processing of the meat harder (3).

The colour of meat is an important quality factor influencing the consumers' choice and it is known to be related to the characteristics such as water retention capacity, texture and chemical composition (5).

Broiler breasts with low L* value and low pH measured 24 hours after slaughter have a pale color and low water retention capacity (6). It has been reported that if the L* value of the broiler breast meat is larger than 53 it can be ranked as lighter coloured and more juicy than normal, as normal

between 48-53, and as dark coloured, when the value is lower than 46 (7).

Some researchers (8) report that, depending on the stress, meat is darker in chickens and higher in pH. Conversely, in some studies (9), were dark-colored poultry meats without depending on pH values. These meats have lower L (brightness) value, higher a (redness) and b (yellowness) values.

Since medicinal plants contain antioxidants, they have a protective effect in nutrients (10). Synthetic antioxidants such as butyl hydroxy anisole and butyl hydroxy toluene have a field of use in meat and meat products, but due to concerns arising from the possible negative effects of such products on human health, there is an ongoing need for healthy antioxidant substances. Therefore, studies on the use of plant extracts are increasing because of the reliability and ease of availability (11).

MATERIALS and METHODS

Animals, Feeds and Experimental Design

In the study, 7 days old, 300 Japanese quail (*Coturnix coturnix japonica*) were used for 28 days. The chicks were divided into 6 groups each consisting of 5 replications and 50 chicks. In the experiment, in addition to the control group, intensive stocking density was established with intensive stocking control group (YYS-KONT) only with basic ration, and basic ration 10 mg / kg Avilamycin supplement YYS-ANT) group and zahter group (YYS-T1, T2, T3) (200, 400, 600 mg / kg essential oil supplemented respectively).

In the study, the quails in the control group were placed in a cage area of 160 cm²/quail in 50x100 cm size cages and the intensive stocking group in a 90 cm²/quail cage. A 24-hour lighting program and nutrient content of commercial feed broiler chick starter was used as shown in Table 1.

The experimental protocol was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Authorization Number: 2012 / 09-03).

Table 1. Ingredient and composition of all the basal diet. (g/kg).**Tablo 1.** Bazal rasyon besin madde ve içerikleri (g/kg).

| Ingredients | Composition |
|-------------------------------------|-------------|
| Maize | 515.0 |
| Wheat | 77.0 |
| Wheat bran | 45.0 |
| Extracted soybean meal | 275.0 |
| Fish meal | 55.0 |
| Vegetable oil | 15.0 |
| Limestone | 10.0 |
| Dicalcium phosphate | 7.5 |
| Sodium chloride | 2.5 |
| Vitamin-mineral premix* | 5.0 |
| Calculated nutrients | |
| ME, (MJ kg ⁻¹)** | 12.6 |
| Crude protein (g kg ⁻¹) | 221 |
| Ca (g kg ⁻¹) | 9.0 |
| P (g kg ⁻¹) | 6.0 |
| Lysine (g kg ⁻¹) | 11.0 |

*: Per kilogram vitamin; retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; α -tocopherol acetate, 1.25 mg; menadione (menadione sodium bisulphate), 1.1 mg; thiamine (thiamine mononitrate), 1.1 mg; riboflavin, 4.4 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; pyridoxine, 2.2 mg; folic acid, 0.55 mg; cyanocobalamin, 0.02 mg; Mn, 74 mg (from MnO); Zn, 45 mg (from ZnO); Cu, 4 mg (from CuO); Fe (from FeSO₄), 12.5 mg; I (from KI), 0.3 mg; Se (from NaSe), 0.15 mg. ** ME: Metabolizable energy. The ME, crude protein, calcium, phosphorus and lysine contents were calculated based on their tabular values listed for the feeding ingredients (12).

*: Kg başına düşen vitamin premiksleri: retinil asetat, 1.8 mg; kolekalsiferol, 0.025 mg; α -tokoferol asetat, 1.25 mg; menadion (menadion sodyum bisülfat), 1.1 mg; tiamin (tiamin mononitrat), 1.1 mg; riboflavin, 4.4 mg; niacin, 35 mg; Ca-pantotenat, 10 mg; piridoksin, 2.2 mg; folik asit, 0.55 mg; Siyanokobalamin, 0.02 mg; Mn, 74 mg (MnO); Zn, 45 mg (ZnO); Cu, 4 mg (CuO); Fe (FeSO₄), 12.5 mg; I (KI), 0.3 mg; Se (NaSe), 0.15 mg. ** ME: Metabolize olabilen enerji. ME, ham protein, kalsiyum, fosfor ve lizin içerikleri, besleme bileşenleri için listelenen tablo değerlerine dayanarak hesaplandı.

Plant Material

The essential oils were extracted from *Thymbra spicata* L. var. *spicata* plant. Blooming plants were collected from their localization and were dried at 35°C.

Characterization of Essential Oil

Determination of the essential oil's chemical ingredient was performed with Thermo Scientific ISQ Single Quadrupole model gas chromatograph. TG-

Wax MS-A model, 5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter x 30 m length, 0.25 μ m film thickness column was used. Helium (99.9%) was used as the carrier gas at a flow rate of 1 mL / min. The ionization energy was set at 70 eV and the mass range m / z at 1.2-1200 amu. For collecting dataScan mode (Scan Mode) was used.

The MS transfer line temperature was 250 °C, the MS ionization temperature was 220°C, the injection port temperature was 220 °C, the column temperature was initially 50 °C and the temperature was increased up to 220 °C at a rate of 3 °C/min. The structure of each compound was defined using mass spectra by Xcalibur program.

Determination of Some Blood Parameters and Antioxidant Potential

At the end of the experiment, 20 quails -10 females and 10 males-were randomly selected from each repeat group and the blood samples were taken during the cervical dislocations (vena jugularis) and after being centrifuged at 3000 rpm for 10 minutes, serum samples were taken into eppendorf tubes and stored at -18°C until the analyzes were carried out.

Analyzes of albumin, total cholesterol, creatine, total protein, triglyceride, HDL, LDL, globulin, urea were performed using Architect C8000 auto analyzer (ABBOTT, Germany) in an accredited laboratory.

Determination of Meat Quality Parameters

In the experiment, a total of 72 quails (6 females and 6 males for each group) were randomly selected and the weight of hot carcass was immediately weighed after the slaughtering was completed. Later, these carcasses were kept in the refrigerator at + 4°C for 24 hours to determine the cold carcass weights. Briskets (M. Pectoralis major), from the right half of each carcass was used to determine pH and color characteristics. After slaughtering, the pH was determined from the brisket by a portable pH meter (Mettler Toledo SG2) with glass electrode (Inlab 427). Glucose (L*), redness (a*) and yellowness coordinates (b*) were determined for color analysis

with the aid of a colorimeter (Konica Minolta CR-400) from the skin 4 hours after slaughter. The following formula was used for determination the carcass yield in the experiment.

Carcass yield (%) = Carcass weight (g) / Live weight (g) * 100

Statistical Analysis

SPSS 11.5 package program was used for statistical analysis (13). The two-way ANOVA test was used to compare groups. In order to determine the difference between the groups, Duncan's multiple comparison test was used. When the results were evaluated statistically, the significance was based on $P < 0.05$. The chi-square test was used to determine the death rates and rates of the research groups.

RESULTS

The essential oil's chemical ingredient is given in Table 2. Thyme oil mainly consisted of carvacrol (71.6%), ocimene (9.03%) and γ -terpinene (5.83%) respectively; and also formed high level of phenolic components including 72% of phenol and 21% of hydrocarbon.

Table 2. Chemical components *Thymbra spicata* L. oil.
Tablo 2. *Thymbra spicata* L. uçucu yağının kimyasal bileşenleri.

| Retention Time (RT) | Rate (%) | Components |
|---------------------|----------|-----------------------|
| 18.04 | 0.67 | l-Phellandrene |
| 18.62 | 0.45 | Delta.3-Carene |
| 22.26 | 0.18 | Succinaldehyde |
| 22.64 | 0.77 | Beta-Myrcene |
| 24.86 | 1.06 | Alpha-Humulene |
| 25.59 | 9.03 | o-Cymene |
| 25.83 | 0.36 | Cis-D-Dihydrocarveol |
| 28.31 | 5.83 | Gama-Terpinene |
| 29.36 | 0.26 | Trans-Sabinenehydrate |

| | | |
|-------|-------|--|
| 31.12 | 0.41 | Cis-Sabinenehydrate |
| 33.99 | 0.08 | 3-Pinanylamine |
| 34.18 | 0.93 | 4-Terpineol |
| 36.01 | 0.31 | Z,Z,Z-1,4,6,9-Nonadecatetraene |
| 36.93 | 0.27 | Thymol |
| 37.12 | 71.62 | Carvacrol |
| 39.39 | 1.91 | Caryophyllene |
| 39.71 | 0.18 | Farnesol |
| 40.01 | 0.11 | Trans-Z-alpha-Bisaboleneepoxide |
| 40.26 | 0.44 | Beta-Lactose |
| 40.58 | 0.33 | Tetraacetyl-d-xlonicnitrile |
| 41.77 | 4.75 | 1Monolinoleoyglyceroltrimethylsilylether |
| 42.30 | 0.09 | 12,15-Octadecadiynoicacid, methylester |
| 42.44 | 0.53 | Caryophylleneoxide |
| 47.51 | 0.42 | Methylperfluorobutyrate |

Serum MDA Level and Some Biochemical Parameters

Stocking density significantly affected serum MDA levels in research groups ($P < 0.01$) (Table 3). The highest serum MDA level (13.36 ± 0.39) was found in negative stocking density control group (YYS-KONT) while the lowest value (10.24 ± 0.65) in positive control group (NYS-KONT) ($P < 0.01$). There was no statistical difference between the study groups in terms of some examined biochemical parameters ($P > 0.05$). Although there was no statistical difference between the groups in terms of other biochemical parameters examined, YYS-KONT serum total albumin (1.04 ± 0.03), globulin (1.63 ± 0.28) creatine (0.29 ± 0.01), urea (1.89 ± 0.28) and total oxidant potential (10.30 ± 1.73) levels, in parallel with serum MDA level, were found numerically higher than the other groups.

Table 3. Serum MDA and some biochemical parameters of the groups.**Tablo 3.** Grupların serum MDA ve bazı biyokimyasal parametre değerleri.

| | NSD-CONT | HSD- CONT | HSD-ANT | HSD-T1 | HSD-T2 | HSD-T3 | |
|--------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|-------------------------|-------|
| SERUM MDA (µmol/L) | 10.24±0.65 ^c | 13.36±0.39 ^a | 11.23±0.65 ^b | 12.25±0.43 ^{ab} | 12.33±0.41 ^{ab} | 10.58±0.34 ^c | ** |
| Albumin (g/dL) | 0.92±0.07 | 1.04±0.03 | 0.94±0.05 | 0.99±0.03 | 0.95±0.52 | 0.93±0.04 | NS/ÖD |
| Total Kolesterol (mg/dL) | 181.71±12.03 | 217.57±10.33 | 186.89±8.64 | 196.14±10.17 | 185.91±11.50 | 181.74±11.80 | NS/ÖD |
| Kreatin (mg/dL) | 0.25±0.01 | 0.29±0.01 | 0.25±0.01 | 0.27±0.01 | 0.26±0.01 | 0.26±0.01 | NS/ÖD |
| TAS (mmol/L) | 1.58±0.12 | 1.98±0.09 | 1.86±0.05 | 1.79±0.09 | 1.58±0.07 | 1.68±0.08 | NS/ÖD |
| TOS (umol/L) | 10.19±0.96 | 10.30±1.73 | 9.17±0.63 | 7.14±0.55 | 7.91±0.71 | 8.54±1.67 | NS/ÖD |
| Total Protein (g/dL) | 2.28±0.16 | 2.68±0.08 | 2.4±0.13 | 2.51±0.09 | 2.50±0.14 | 2.37±0.02 | NS/ÖD |
| Trigliserit (mg/dL) | 188.63±14.93 | 264.99±16.93 | 233.24±17.71 | 225.99±23.37 | 190.39±14.38 | 194.94±14.34 | NS/ÖD |
| HDL (mg/dL) | 84.45±8.39 | 109.73±7.96 | 91.65±7.19 | 100.16±6.71 | 91.66±7.93 | 86.92±6.97 | NS/ÖD |
| LDL (mg/dL) | 59.76±10.41 | 54.84±6.08 | 51.14±9.85 | 44.04±4.19 | 59.23±11.32 | 59.87±17.52 | NS/ÖD |
| Globulin (mg/dL) | 1.35±0.09 | 1.63±0.06 | 1.45±0.09 | 1.51±0.06 | 1.55±0.09 | 1.43±0.07 | NS/ÖD |
| Urea (mg/dL) | 1.15±0.36 | 1.89±0.28 | 1.78±0.31 | 1.69±0.26 | 1.60±0.31 | 1.45±0.39 | NS/ÖD |

** The difference between the averages indicated by different letters on the same line is statistically significant (P <0.01). NS: Not significant

** Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklılık istatistik olarak önemlidir (P<0.01). ÖD: Önemli değil

Meat Quality Parameters

It was found that meat quality parameters and pH are significantly influenced by the treatments (P<0.05) (Table 4). It was found that high stocking density reduces the meat pH values and this reduction was more pronounced especially in the

antibiotic supplement group (YYS-ANT, 5.97 ± 0.04) and the thyme essential oil supplement groups YYS-T1 (6.02 ± 0.04) and YYS-T2 (6.06 ± 0.03). L *, a * and b * values of the meat quality parameters of the groups were not affected by the treatments.

Table 4. Meat quality parameters.**Tablo 4.** Et kalite parametreleri.

| Meat pH Values | | | | | | | |
|----------------------|------------------------|-------------------------|------------------------|------------------------|-------------------------|-------------------------|-------|
| | NSD-CONT | HSD- CONT | HSD-ANT | HSD-T1 | HSD-T2 | HSD-T3 | |
| pH 0 | 6.23±0.18 ^a | 6.08±0.05 ^{bc} | 5.97±0.04 ^c | 6.02±0.04 ^c | 6.06±0.03 ^{bc} | 6.16±0.03 ^{ab} | ** |
| Meat Colour Analysis | | | | | | | |
| 24'th hour L | 55.73±0.68 | 55.83±0.58 | 55.01±0.80 | 55.37±0.72 | 54.23±0.72 | 54.95±0.64 | NS/ÖD |
| 24'th hour A | 10.40±0.75 | 10.89±0.63 | 11.13±0.74 | 10.44±0.73 | 10.64±0.61 | 11.43±0.63 | NS/ÖD |
| 24'th hour B | 7.48±0.40 | 7.70±0.39 | 8.01±0.45 | 8.30±0.58 | 6.96±0.37 | 7.58±0.42 | NS/ÖD |

The differences between averages with different letters on the same line are important.

** The difference between the averages indicated by different letters on the same line is statistically significant (P<0.01).

NS: Not significant

Aynı satırda farklı harfler taşıyan ortalamalar arasındaki farklılıklar önemlidir.

** Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklılık istatistik olarak önemlidir (P<0.01).

ÖD: Önemli değil

DISCUSSION and CONCLUSION

In the study, the antioxidant potential of thyme (zahter) (*Thymbra spicata* L. var. *Spicata*) and its impacts on meat quality of Japanese quails reared in different stocking densities were investigated. MDA values of YYS-T3 group were found to be closer to that of the control group (10.58 $\mu\text{mol/L}$ ml and 10.24 $\mu\text{mol/L}$ respectively). Serum MDA levels of all other treatment groups quails, except YYS-T3, were found significantly higher compared to the control group. The findings are consistent with Malayoğlu et al. (14) studied 100 mg carvacrol + 250 mg carnosic acid additive effects. Kaya and Turgut (15) investigated the effects of mint, thyme, sage extracts and vitamin E on triglyceride and serum cholesterol levels. These supplements were added to egg hen ratios at different doses and thyme and sage extracts of 300 mg / kg could be used as an alternative to vitamin E due to lipid oxidation inhibition and cholesterol-lowering effects of plant extracts and vitamin E additions.

No statistical significance was found in serum creatinine, total cholesterol, HDL and LDL values in essential oil-treated groups and these results were parallel with Seven et al. (16) studied Japanese quails breeding in stocking densities. However, Seven et al. (16) found that stocking density groups' total protein, serum albumin and globulin levels were higher than of the control group and the serum urea levels in stocking density groups were higher than the other groups and these findings are inconsistent with this study.

Seven et al. (16) and Chowdhury et al. (17), suggested that serum protein levels are effective on the immune system. In case of stress-induced disease, toxicity, etc. an increase can be observed in these values due to deterioration of immunity system and the significant increase in serum albumin, globulin, urea, total protein levels are related to the oxidative stress caused by stocking density. Aksu et al. (18) reported that using thyme essential oil (200-400-600 mg / kg) caused no significant difference in

cholesterol and TAS levels, but there was no significant difference in triglyceride and TOS averages in 400 mg / kg group.

Yörük et al. (19) showed that when stocking density increased, triglyceride, total protein and creatine levels in blood serum parameters of Japanese quails decreased significantly. The decrease of triglyceride and glucose levels in accordance with the increase of stocking density indicated that the consumption of feed might have reduced as the stocking density intensified. Since it was seen that the the amount of feed consumption in the experimental groups was not decreased due to the stocking density, the cause of decline in triglycerides and glucose remained unexplained.

Depending on the changes on hemoglobin and myoglobin concentration, a colour change occurs in meat and this change is correlated with the pH value of meat. If the meat colour is dark, the pH is high; if it is lighter, the pH is recorded as low (20).

The values obtained for the L, a and b color features of briskets were not statistically significant. This is consistent with the studies reporting stocking density has no effect on color features (21). Meluzzi et al. (22) reported that the stocking density in broilers significantly increased the a * and b * values of meat. Zhang et al. (23) reported that brisket color characteristics were not affected by the stocking density. Castellini et al. (24) reported that the stocking density increased significantly especially L * and b * values of broilers bred at different stocking densities.

In current study, essential oil had a significant effect on breast meat. Ph values of experimental groups, 200 mg / kg essential oil group (YYS-T1) and antibiotic (YYS-ANT) group, had similar pH values and both groups had higher values than the other groups. In some studies, it was reported that the stocking density had no effect on meat pH values (21, 25).

At the end of the study, it was determined that high stocking density (90 cm / quail) increased

the stress level in quails (significantly increased the serum MDA level) and supplementation of thyme essential oil enhanced the serum MDA levels comparing to the negative control group. Thyme essential oil had no significant effect on other biochemical parameters and it was determined that stocking density causes a tendency to increase serum albumin, globulin, creatine, urea levels and the total oxidant potential.

As a result, it was concluded that thyme essential oil addition into the diet of Japanese quails under the stocking density treatment, was more effective in alleviating the adverse effects caused by stocking density, especially in 600 mg / kg of dosage.

Conflict of interest

The authors declare that they have no conflict of interest.

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