


Effects of Supplementation with Rosemary (*Rosmarinus officinalis* L.) Volatile Oil on Growth Performance, Meat MDA Level and Selected Plasma Antioxidant Parameters in Quail Diets ^[1]

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Abstract

The current study was conducted to evaluate the effects of dietary supplementation with rosemary volatile oil on performance, meat quality and selected plasma antioxidant parameters of quails. A total of 192 1-day-old Pharaoh (*Coturnix coturnix Pharaoh*) quails, including both males and females, were divided into three groups containing 64 quails and treated as follows: (1) a control group with 0 mg volatile oil/kg of diet; (2) 200 mg/kg rosemary volatile oil plus-RVO1; and (3) 250 mg/kg rosemary volatile oil plus-RVO2. The diets were prepared fresh for each treatment. The experiment was carried out for 42 days. Dietary treatments did not have any significant effect on live weight gain, feed intake, feed conversion rate, hot and cold carcass yield. There were significant differences for the plasma MDA level ($P<0.01$) and meat MDA level ($P\leq 0.001$) between the control and treatment groups. Plasma SOD activity not affected by addition of rosemary volatile oil but plasma GPx level was significantly ($P<0.01$) affected by dietary treatments. In conclusion, rosemary volatile oil may be a potential natural antioxidant for quails and used to retard lipid oxidation in animal diets to improve meat products quality and animal performance.

Keywords: Antioxidant, Performance, Quail, Rosemary, Volatile oil

Bıldırcın Rasyonuna Biberiye Uçucu Yağ İlavesinin Büyüme Performansı, Et MDA Düzeyi ve Bazı Plazma Antioksidan Parametreleri Üzerine Etkisi

Özet

Bu çalışma, rasyona ilave edilen biberiye uçucu yağının, bıldırcınlarda performans, et kalitesi ve bazı plazma antioksidan parametreleri üzerine etkilerini değerlendirmek amacıyla yapıldı. Araştırmada toplam 1 günlük yaşta 192 adet Pharaoh (*Coturnix coturnix Pharaoh*) erkek ve dişi bıldırcınlar kullanıldı. Araştırmada her bir deneme grubunda 64 bıldırcın olmak üzere toplam 3 ana gruba bölündü ve deneme grupları sırasıyla: (1) rasyona 0 mg/kg biberiye uçucu yağı; (2) 200 mg/kg biberiye uçucu yağı ve (3) 250 mg/kg biberiye uçucu yağı ilave edildi. Rasyonlar her deneme grubu için taze olarak haftalık olarak hazırlandı. Deneme 42 gün boyunca yürütüldü. Araştırmada rasyona biberiye uçucu yağ ilavesinin canlı ağırlık artışı, yem tüketimi, yemden yararlanma oranı, sıcak ve soğuk karkas verimi üzerine önemli bir etkisi tespit edilmedi. Araştırmada plazma MDA ($P<0.01$) ve et MDA ($P\leq 0.001$) düzeyinde kontrol ve deneme grupları arasında önemli farklılıklar belirlendi. Çalışmada plazma SOD aktivitesi biberiye uçucu yağ ilavesinden önemli düzeyde etkilenmemiş fakat plazma GPx oranını önemli ($P<0.01$) düzeyde etkilemiştir. Sonuç olarak, bıldırcın rasyonlarına ilave edilen biberiye uçucu yağının lipid oksidasyonu geciktirerek potansiyel bir doğal antioksidan katkısı olabileceği ve hayvan performansını iyileştirmek amacıyla kullanılabileceği sonucuna varılmıştır.

Anahtar sözcükler: Antioksidan, Performans, Bıldırcın, Biberiye, Uçucu yağ

INTRODUCTION

Recently, aromatic plants and plant extract products have received attention also in their useful physiological

functions and antioxidant activity. The oxidative deterioration of lipid and proteins is a major concern for food technologists due to the loss of quality associated with those processes. Lipid oxidation decreases nutritional



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and sensory properties of foods since it involves the loss of essential fatty acids and vitamins and generation of toxic compounds, causing additionally, flavour, texture and color deterioration [1]. Oxidative deterioration of lipids is an important factor limiting the shelf life of foods. Use of antioxidants can minimize the degree of lipid peroxidation [2]. To prevent or delay this autoxidation process, traditional antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) have been used for more than five decades. However, these synthetic antioxidants (BHT, BHA and TBHQ) are known to have toxic and carcinogenic effects on human health. Therefore, we need to highly sensitive to lipid peroxidation and free radicals form research for alternative antioxidants. In particular, the extracts of many members of the *Labiatae* (*Lamiaceae*) family (oregano, marjoram, savory, sage, rosemary, thyme, and basil), which are antioxidative, have a high total phenol content. Among these, rosemary and sage have been widely used and most of their antioxidant components have been identified [3]. Dorman et al. [4] observed that, while these antioxidant characteristics are not completely concerned with the total phenolic contents, they do appear to be strongly conditional on rosmarinic acid, the major phenolic component present.

Rosemary is currently a widely used aromatic plant which has been recognized to have high antioxidant activity [5]. The objects associated with the antioxidant activity of rosemary are the phenolic diterpenes, such as carnosol, rosmanol, 7-methyl-epirosmanol, isorosmanol and carnosic acid, and the phenolic acids, such as rosmarinic and caffeic acids. Conversely, the major constituents of rosemary volatile oil are monoterpenes such as α -pinene, myrcene, 1,8-cineole and borneol. These components possess strong antibacterial, antifungal, antiviral and antimicrobial activities [6]. However, the properties of the volatile oils have been found to differ rely on extraction method used and different varieties of rosemary, grown in different area under different conditions may vary in the content of these phenolic compounds. So, the present study was aimed to determine the effect of rosemary volatile oil on quail performance, the susceptibility of raw breast meat lipid oxidation and selected plasma antioxidant parameters.

MATERIAL and METHODS

Animals, Diets and Experimental Design

A total of 192 1-day-old Pharaoh (*Coturnix coturnix Pharaoh*) quails, including both males and females, were divided into three groups containing 64 quails each group was randomly divided into four subgroups comprised of 16 quails each. Groups as follows: (1) a control group with 0 mg volatile oil/kg of diet; (2) 200 mg/kg rosemary volatile oil -RVO1; and (3) 250 mg/kg rosemary volatile oil

-RVO2. Animals were obtained from the Uludag University Animal Health and Production, Research and Application Centre for quail breeding (Bursa, Turkey). In addition, this study was conducted under an approved protocol by Animal Care and Use Committee of University of Uludag (Approval number: 14.01.2010, 2010-01/108). The diet was fed to the quails in the form of mash and water *ad libitum* throughout the entire experimental period (42 d). Newly hatched chicks in all of the groups were reared under the same growing conditions in brooding cages (colony type) in an open-sided house with mechanical ventilation. The quails were transferred randomly at the fourth week of age from growing cages to laying cages (100 cm wide, 45 cm deep, 21 cm high in front, and 17 cm high in the rear, 112.5 cm² per quail) and housed there until the end of the study. All the chicks were brooded and reared at 28°C for the 1st wk, 27°C for the 2nd wk, 24°C for the 3rd wk, and 18-21°C from the 28th day until the quails reached 42 days of age. The quails received a basal diet (maize and soy bean based; 22.1% crude protein; 12.5 MJ/kg metabolisable energy) that was formulated to meet the NRC [7] requirements for nutrients including vitamins and minerals. The diet did not contain antibiotics, coccidiostats or growth promoters. The content of the basal diet for meat quails is presented in Table 1. Group feeding was used in all treatment groups.

Table 1. Ingredients and chemical composition of the basal diet

Ingredients for Meat Quail	g/kg
Corn	473.0
Soybean meal	360.0
Wheat	50.0
Corn gluten	50.0
Vegetable oil	30.0
CaCO ₃	16.0
DCP	6.2
Salt	3.0
L-Lysine	3.5
D-L methionine	4.8
Vit-min premix ^a	3.5
Calculated nutrient concentration	
Metabolisable energy ^b MJ/kg	12.49
Crude protein %	22.12
Ether extract %	7.67
Ash %	5.64
Dry matter %	90.75
Starch %	33.88
Sugar %	55.98

^a Provides (mg per kg diet): retinol, 2.4 mg; cholecalciferol, 0.075 mg; α -tocopherol acetate, 20 mg; thiamin, 3 mg; riboflavin, 3 mg; pyridoxal, 3.5 mg; cobalamin, 0.01 mg; niacin, 20 mg; pantothenic, 4 mg; folic acid, 1 mg; choline, 600mg; biotin, 0.03 mg; Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; I, 1mg; Co, 0.2 mg; Se, 0.15 mg; ^b Metabolisable energy content of diets was estimated using the equation devised by Carpenter and Clegg [11]

The volatile oil (VO) dosages added to diet were chosen based on information from the literature and from the effective dosage determined by a previous study [8,9]. A 24-h constant lighting program was also maintained throughout the experimental period.

The nutritional composition of the diets was determined according to the Association of Official Agricultural Chemists [10]. The metabolisable energy (ME) levels of diets were estimated [11] using the following equation from Carpenter and Clegg:

$$\text{ME, kcal/kg} = 53 + 38 [(\text{Crude Protein, \%}) + (2.25 \times \text{ether extract, \%}) + (1.1 \times \text{starch, \%}) + (1.05 \times \text{sugar, \%})].$$

Performance Parameters of Quail Chicks

The quails were weighed individually at the beginning of the experimental period, after which the animals were weighed weekly to calculate live weight gain (LWG). Mortality was recorded when it occurred. Feed consumption was recorded weekly and expressed as g per quail per week. The feed conversion rate (FCR) was calculated as kg feed per kg body weight gain. At the end of the experimental period, the sex ratio was established in each group and 20 male quails from each group (5 male quails from each replicate) were randomly selected and weighed to determine the carcass yields (CY). The inert organs, heads and feet were removed after the carcasses were passed through a poultry defeathering machine. The chilled carcass weights were determined after incubation at 4°C for 18 h, and then the CY was calculated.

Determination of VO in Rosemary

The pure VO of rosemary (GC-MS tested, origin Mersin/Turkey, Semi Eterik Yağ Sanayi ve Dış Ticaret Ltd. Şti.) steam distillation extraction were obtained from a volatile oil company. Gas chromatography analysis was carried out on a Mass Spectrometry (HP 6890 Series Gas Chromatograph 5973 Mass Selective Detector System China - Agilent HP-Innowax capillary column) (60 x 0.25 µm x film thickness 0.25 µm). The temperature was programmed to rise from 60°C to 220°C at 4°C/min. The injection was performed at 250°C in split mode. Helium gas was used as a carrier at 1.3610 atm. The detection was performed by FID at 250°C, and the injection volume for all samples was 0.1 µL. Chromatograms were determined using mass spectrometer (MS) or MS/MS. The data was calculated using internal standards (Wiley GC/MS library). Volatile oil compositions of rosemary plant is shown in Table 2.

TBA Analysis of Meat Samples

Malondialdehyde (MDA) was measured as a secondary oxidation product according to the TBA method described by Tarladgis et al. [12] using spectrophotometry with some modifications. At the end of the experimental period, 30 breast-meat samples (10 samples from each group)

Table 2. Volatile oil components of rosemary (*Rosmarinus officinalis*)

Components	(%)
α-pinene	11.77
Camphene	5.21
β-pinene	1.29
Limonene	1.89
1,8-cineole (Eucalyptol)	51.63
Camphor	3.11
Borneol	4.16
Caryophyllene oxide	0.75
Bornyl acetate	2.56
Sabinene	0.54
β-myrcene	0.85
p-eymene	3.12
Linalool	0.63
β-caryophyllene	3.78
α-terpineol	2.73
Carvacrol	1.03

were subjected to TBA analysis. The lipid oxidation value of breast-meat samples stored at +4°C was determined on days 3 and 7 post slaughter. A modification of the 2-thiobarbituric acid method was used, and the results are expressed as the amount of 2-thiobarbituric acid reactive substances (mg MDA). This method is based on the observation of a red colour that is created by the oxidation of unsaturated fatty acids with TBA after heating with MDA. For the analyses, a 10 g sample was homogenised with distilled water in a blender and transferred to a Kjeldahl flask where it was distilled to distillate aggregation by adding 2.5 mL 4 N HCl (Merck, Germany) and 1 mL Antifoam A. The reactant, 5 mL TBA (Merck, Germany), was added to 5 mL distillate and incubated in a boiling water bath for up to 30 min. The final solution and a blank were measured in a spectrophotometer at 538 nm. The obtained absorbance value was multiplied by 7.8. The final value was expressed as mg MDA per kg sample.

Blood Analyses

Blood samples (2 mL) were collected by venipuncture into an Etilen Diamin Tetra Aceticacid (EDTA) tube during slaughter time (42th day). Plasma was separated by centrifugation at 3.000 × g for 10 min. and placed in separate eppendorf tubes then stored at -80°C until analyses day. SOD activity was measured by using the assay kit (BioVision Research Products, Mountain View, USA, cat. no. K335-100). The sensitive SOD assay kit utilizes WST-1, which produces a water-soluble Formosan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidize activity and is inhibited by SOD. Therefore, the inhibition activity of SOD can be determined using a

colorimetric method. The results were expressed as the inhibition rate (%). GP_x activity was measured by using GP_x colorimetric assay kit (BioVision, cat. no. K762-100). All plasma measurements were read in the microplate spectrophotometer (Biotek Epoch, USA). Plasma malondialdehyde (MDA) level was measured the MDA method described by Ohkawa et al.^[13] Method briefly, 100 µL plasma was added to 50 µL of sodium dodecyl sulfate (SDS 8.1%) and then vortexed, incubated for ten minutes at room temperature. 375 µL of acetic acid (pH 3.5, 20%) and 375 µL thiobarbituric acid (0.6%) was added and incubated in a boiling water bath for 60 min. The samples was left to cool to room temperature. 1.25 mL butanol:pyridine (15:1) was added, vortexed and centrifuged at 1000 rpm for five min. Finally, organic pink solution was measured in a spectrophotometer (Shimadzu UV 1601, Kyoto, Japan) at 532 nm. Results were defined nmol/mL for plasma.

Statistical Analysis

All data were subjected to ANOVA using the ANOVA procedure of SPSS (SPSS, 2011). The data were first analysed as a completely randomized design as a random factor

to examine the overall effect of treatments. Effect of rosemary was determined by the "contrast" option of the GLM procedure. When this effect was significant (i.e., $P < 0.05$), orthogonal polynomial contrasts using contrast coefficients that were used to determine linear and quadratic responses to rosemary dosages. The significance of differences among treatments was performed using Dunnett's test.

RESULTS

The ingredients and chemical composition of the basal diet is presented in *Table 1*. Volatile oil compositions of rosemary plant is shown in *Table 2*. The 1-8 cineole (51.63%), α -pinene (11.77%), camphene (5.21%) and borneol (4.16%) were determined to be the main active components for rosemary volatile oil. The effects of dietary treatments on LWG, feed intake, FCR, hot and cold CY were shown in *Table 3*. Dietary treatments did not have any significant effect on LWG, feed intake, FCR, hot and cold CY. Lipid oxidation degree of meat samples (meat MDA level) stored for 3 and 7 day in refrigerator conditions and plasma

Table 3. Effect of Rosemary volatile oil on live weight gain, feed intake, feed conversion rate, hot carcass yield and cold carcass yield

Parameters	n	Period	Groups			P-values
			CG ¹ mean±SD	RVO 1 ² mean±SD	RVO 2 ³ mean±SD	
Live Weight Gain (g)	4	0-3 week	103.63±0.86	105.23±2.31	101.67±1.17	0.16
		4-6 week	98.64±0.98	93.47±2.07	94.07±0.73	1.00
		0-6 week	202.27±0.48	198.71±3.94	195.75±1.48	0.10
Feed Intake (g)	4	0-3 week	285.66±9.88	275.66±3.08	290.61±2.55	0.13
		4-6 week	671.84±16.57	672.68±21.28	671.31±35.32	0.97
		0-6 week	957.50±13.43	948.34±21.53	961.93±36.78	0.73
Feed Conversion rate (feed/gain)	4	0-3 week	2.85±0.037	2.62±0.044	2.75±0.010	0.099
		4-6 week	7.13±0.38	7.21±0.30	6.81±0.18	0.63
		0-6 week	4.91±0.18	4.77±0.15	4.73±0.074	0.67
Hot Carcass Yield (g)	20	End of trial	76.24±0.70	76.03±0.68	77.97±0.47	0.06
Cold Carcass Yield (g)	20	End of trial	74.66±0.76	74.84±0.59	76.38±0.49	0.07

¹ Control group; ² Rosemary volatile oil (Group supplemented with 200 mg/kg rosemary volatile oil); ³ Rosemary volatile oil (Group supplemented with 250 mg/kg rosemary volatile oil)

Table 4. Effect of Rosemary volatile oil on meat and plasma MDA level, plasma SOD activity and plasma GPx level

Parameters	n	Period	Group			P-values
			CG ¹ Mean±SD	RVO 1 ² Mean±SD	RVO 2 ³ Mean±SD	
Meat MDA level (mg/kg meat)	10	A	0.11±0.01 ^a	0.06±0.009 ^b	0.05±0.007 ^b	0.001
		B	0.86±0.01	0.73±0.017	0.64±0.11	0.14
Plasma MDA level (nmol/mL)	20	End of trial	0.24±0.25 ^a	0.14±0.15 ^b	0.20±0.10 ^a	0.002
Plasma SOD(%)	20	End of trial	93.24±1.84	93.90±2.05	92.23±1.77	0.56
Plasma GPx(U/mL)	20	End of trial	0.59±0.04 ^b	0.58±0.03 ^b	0.74±0.03 ^a	0.007

¹ Control group; ² Rosemary volatile oil (Group supplemented with 200 mg/kg rosemary volatile oil); ³ Rosemary volatile oil (Group supplemented with 250 mg/kg rosemary volatile oil) A: storage of 3rd day at +4°C B: storage of 7th day at +4°C; MDA: Malondialdehyde SOD: Superoxide Dismutase GPx: Glutathione Peroxidase Letters; ^a^b in the same row indicate significant differences between different letters

MDA level, SOD activity and GPx levels are given in *Table 4*. Lipid oxidation, as measured by MDA formation, varied ($P \leq 0.001$) between the dietary treatments especially MDA value significant important at 3 day of storage period. Groups treated with rosemary volatile oil had the lowest MDA values. There were significant differences ($P < 0.01$) for the plasma MDA level between the control and RVO 1 group. The lowest plasma MDA level was determined in the group including 200 mg/kg rosemary volatile oil (RVO 1). Plasma SOD activity was not affected by addition of rosemary volatile oil but plasma GPx level was significantly ($P < 0.01$) affected by dietary treatment (RVO 2).

DISCUSSION

Volatile Oil Composition of the Rosemary Plant

In this study the main active components of rosemary volatile oil were determined 1.8-cineole (51.63%), α -pinene (11.77%) and camphene (5.21%). Ghazalah and Ali [15] stated that main active components were camphor (11-16%), pinene (15-20%) and cineole (30-35%). These findings agree with those obtained by Wolski et al. [16] and Porte et al. [17]. Debersac et al. [18] reported that the major component of dried leaves of *Rosmarinus officinalis* (L.) was monoterpene oxide 1-8 cineole (36.1%). Farag et al. [19] stated that these active compounds have high antioxidant activity due to the presence of phenolic groups in their structure. Plants belonging to the *Lamiaceae* family are very rich in polyphenolic compounds. Chemical composition of rosemary volatile oil can vary between regions and it depends mostly on climate, soil composition, plant organ, age, stage of vegetable cycle.

Growth Performance

The addition of rosemary volatile oil in quail diet did not impair LWG, feed intake, FCR and CY (*Table 3*). Performance parameters are generally positively affected by the addition of aromatic herbs and their extracts into diets, and in this study, rosemary volatile oil did not have adverse effects on the performance parameters. However, few studies have shown negative effects on performance parameters when using mixtures of active compounds and volatile oils [20]. Al-Kassie [21] confirmed that adding 0.5 and 1 g/kg anise and rosemary oil, respectively, significantly improved live weight gain and the feed conversion value ($P < 0.05$). Hernandez et al. [22] have demonstrated that adding 5.000 ppm of an herbal mixture of members of the *Labiatae* family such as rosemary can improve live weight gain for 42 d. These results may have derived from the positive effects of aromatic herbs and their volatile oils in the digestive system, where they can improve the activity of enzymes that help in the digestion of feed. Yesilbag et al. [9] determined that the increasing concentration of rosemary oil (140 mg/kg) caused a significant ($P < 0.05$) increase in live weight, live weight gain and carcass yields during the

growing and finishing periods. But feed intake and FCR were not significantly influenced by treatments. Yesilbag et al. [23] explained that the inclusion of rosemary VO at the level of 200 mg/kg to the laying quail diets improved FCR ($P < 0.01$).

Meat MDA Level

The results of quail meat MDA levels are presented in *Table 4*. The effects of dietary treatment on TBA values of refrigerated (+4°C) raw breast meat during different storage times (3 and 7 d) are shown in *Table 4*. Thiobarbituric acid (TBA) analysis is an efficient way to measure antioxidant activity in meat products. This analysis is an indicator of MDA, a product of oxidation; thus, the MDA value increases during the storage period. At the end of this experiment, when the breast meat of the samples were tested, MDA value in the groups including rosemary volatile oil was significantly lower than the control group ($P < 0.001$) especially MDA value significantly low in experiment groups at 3d of storage period. This result indicated that phenolic compounds from rosemary prevent thigh meat from oxidizing. Botsoglou et al. [24] determined that broilers that were given diets enriched in oregano essential oil (50, 100 mg/kg) increased the antioxidative stability of chicken tissue. As such, herbs contain several compounds that can extend the shelf life and improve the quality of meat products [25]. In addition, dietary administration of rosemary and sage essential oil extract to broilers resulted in a decrease in TBA levels from the Day 3 to Day 9 [5].

Biochemical Analyses

The results of quail plasma MDA, SOD and GPx values are presented in *Table 4*. Anti-oxidant enzymes are most effective when acting synergistically with one another or with other components of the anti-oxidant barrier of the organism when their activity remains balanced. It has been shown that nutrition plays a vital role in maintaining the pro-oxidant-antioxidant balance [26]. Antioxidant enzymes such as SOD and GPx are the first line defence antioxidants. In the present study, the GPx value in RVO 2 increased significantly but no statistically significant differences were found for SOD activity the control and the other experimental groups. The MDA level in plasma was reduced ($P < 0.01$) by the inclusion of rosemary volatile oil at 200 mg/kg level. Lin et al. [27] reported that the intakes of herbs in chickens results in an increase in serum antioxidant enzyme activities and decrease in MDA level. Changes in these enzymes could be attributed to the presence of phenolic compounds in the rosemary plant [28]. The substances have strong antioxidant properties, which could protect organisms against oxidative stress. Yesilbag et al. [29] determined that the highest SOD activity value (527.48%) was found in group RVO1, which was fed 100 mg/kg rosemary oil. Overall, oil extract derived from herbal plants could be considered a potential growth promoter for poultry due to its digestive stimulant effect and antioxidant

effects. Additional research is needed to achieve a better understanding of the effects of these oils on plasma antioxidant parameters.

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