

An investigation on the supplementation of rosemary volatile oil to the laying quail diets

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Abstract: The purpose of the current study was to detect the effects of different levels of rosemary volatile oil (VO) supplemented to laying quail diets on performance, egg quality parameters, and egg yolk oxidative stability. In this study, a total of 105 laying quails (*Coturnix coturnix Pharaoh*) at the age of 6 weeks were used and 3 main groups were formed, each containing 35 quails. Also, each main group was separated into 5 replicates containing an equal number of quails. Quails were fed a control diet without rosemary VO or diets which were containing at different levels (200 or 400 mg/kg) of rosemary VO supplemented to the control diet. At the end of the study, which was continued for a total of 56 days, no significant difference was found in the feed intake, feed efficiency and egg weights among the control and experimental groups. The supplementation of rosemary VO at the levels of 200 and 400 mg/kg to the diets caused an increase in quails' egg production ($P<0.01$). However, there was no effect on the exterior and interior egg quality parameters. A significant decrease in malondialdehyde (MDA) levels in egg yolk were observed with both levels of rosemary VO at storage (+4 OC) for 7 ($P<0.001$) and 28 ($P<0.01$) days. Considering these data, it was concluded that rosemary VO can be supplemented to laying quail diets as a natural antioxidant without negatively affecting performance and egg quality parameters.

Keywords: Antioxidant, egg quality, laying quail, malondialdehyde, rosemary volatile oil.

Biberiye uçucu yağının yumurtacı bildircin rasyonlarına ilave edilmesi üzerine bir araştırma

Özet: Bu çalışmanın amacı, yumurtacı bildircin rasyonlarına farklı düzeylerde ilave edilen biberiye uçucu yağının performans, yumurta kalite parametreleri ve yumurta sarısının oksidatif stabilitesi üzerindeki etkilerini belirlemektir. Araştırmada, 6 haftalık toplam 105 yumurtacı bildircin (*Coturnix coturnix Pharaoh*) kullanılmış ve her biri 35 hayvan içeren 3 ana grup oluşturulmuştur. Ayrıca, her ana grup eşit sayıda bildircinden oluşan 5 tekrar grubuna ayrılmıştır. Bildircinler, biberiye uçucu yağı içermeyen kontrol rasyonu veya kontrol rasyonuna ilaveten biberiye uçucu yağının farklı düzeylerini (200 veya 400 mg/kg) içeren deneme rasyonları ile beslenmiştir. Toplam 56 gün sürdürülen araştırmanın sonunda, kontrol ve deneme gruplarının yem tüketimi, yemden yararlanma ve yumurta ağırlıklarında önemli bir fark bulunmamıştır. Biberiye uçucu yağının rasyona 200 ve 400 mg/kg düzeylerinde ilave edildiği deneme gruplarının yumurta üretiminde artış ($P<0,01$) saptanmıştır. Yumurtaların buzdolabında depolanmasının (+4 OC) 7. ($P<0,001$) ve 28. ($P<0,01$) günlerinde, biberiye uçucu yağının her iki dozu da yumurta sarısı malondialdehit seviyelerinde önemli bir düşüşe yol açmıştır. Bu veriler göz önüne alınarak; biberiye uçucu yağının bildircinlerin performans ve yumurta kalite parametrelerini olumsuz etkilemeksizin, rasyonlara doğal bir antioksidan olarak ilave edilebileceği sonucuna varılmıştır.

Anahtar sözcükler: Antioksidan, biberiye uçucu yağı, malondialdehit, yumurtacı bildircin, yumurta kalitesi.

Introduction

The ban on the supplementation of antibiotics to poultry feeds has inspired interest in natural feed additives such as probiotics, prebiotics, aromatic herbs and their essential oils which are not causing residue in animal products and resistance in microorganisms. The antimicrobial (18, 20, 41, 49), antioxidant (10, 35, 51), antifungal (33, 39), antiparasitic (44, 45), antiviral (8),

anti-inflammatory (1), hypolipidemic (54), immunostimulatory (1, 54), digestive system stimulator (46) and digestive enzyme activity enhancer (54) effects of herbal volatile oils are proved with many scientific studies. Rosemary (*Rosmarinus officinalis L.*), aromatic herb specific to the Mediterranean climate, is now widely grown all over the world. It is known as the herb with the highest antioxidant activity among aromatic herbs (19,

38). This effect of rosemary is derived from the phenolic compounds, carnosic acid, carnosol, camphene, ursolic acid, rosmarinic acid, 1,8-cineole, α pinene and other VO components that give the herb its characteristic odour (3, 6, 48, 50). Viuda-Martos et al. (57) tested volatile oils obtained from five aromatic herbs (oregano, thyme, clove, sage and rosemary) commonly used in the Mediterranean diet for their antioxidant properties using different in vitro methods and concluded that rosemary VO has the highest iron-chelating ability.

Recently, there has been a strong debate about the safety aspects of chemical preservatives, as they are thought to be responsible for a number of residual toxicity, carcinogenic and teratogenic cases. Therefore, there has been a heavy demand for foods containing natural preservatives around the world. In line with this demand, the researchers focused on studies to determine the protective effect of natural additives, such as extracts from different herbs, to extend the shelf life of foods. Several studies have identified many beneficial effects of rosemary, including its potent antioxidant activity (48, 57) as well as antibacterial, anti-inflammatory and immunomodulatory effects (15, 23, 24, 60). Our hypothesis was that the strong antioxidant effect of rosemary VO would extend the shelf life of quail eggs and improve the quail's performance due to the general effects of herbal volatile oils (increasing the flavor of the feed and the activity of digestive enzymes, etc.). Thus, the purpose of the current study was to investigate the effects of different levels of rosemary VO on performance, exterior-interior egg quality parameters in laying quails and also to prove the antioxidant activity by determining the effect on the oxidative stability of the eggs.

Materials and Methods

Quails, housing and management: This research was conducted within the framework of the rules determined by the Uludağ University Ethical Committee (decision no. 2018-15/04). In this study, a total of 105 quails (*Coturnix coturnix Pharaoh*) at the age of 6 weeks were used and 3 main groups were constituted, each containing 35 quails. Also, in order to compare the feed intake and feed efficiency parameters of the experimental groups, each main group was divided into 5 replicates containing 7 quails. The quails were placed in cages at 112.5 cm² per quail and kept there until the end of the study. Feed and water were provided *ad libitum* to quails. They were exposed to 16 h of light and 8 h of darkness a day. Average ambient temperature was 22 °C and the relative air humidity was 65 %. The study was continued for 56 days.

Experimental diets and measurements: The quails were fed a corn and soy-based basal diet formulated to meet the NRC nutrient requirements of the laying quails

(42). The diet did not contain any antioxidant other than a basic level of vitamin E (Table 3). Quails were fed a control diet without rosemary VO or diets containing rosemary VO at levels of 200 mg/kg (Group 1) or 400 mg/kg (Group 2) in addition to the control diet. While deciding on the levels of rosemary VO supplemented to the treatment group, the information in the literature (22, 59) were taken into consideration. The specific gravity value of rosemary VO was detected by the supplier company (Semi Ethernic Oil Ind.trade.co.ltd., Mersin /Turkey). This value were considered for calculating the amount of rosemary VO to add to the diets. The nutrient composition of the basal diet was detected according to AOAC procedures (4). Spectrophotometric determinations of calcium and total phosphorus were made according to the methods of Farese et al. (21) and, Gericke and Kurmies (26), respectively. Carpenter and Clegg equation (14) was used to calculate the metabolizable energy level: ME, kcal/kg = 53+ 38 [(CP, %) + (2.25 x ether extract, %) + (1.1 x starch, %) + (sugar, %)].

Performance, exterior and interior egg quality:

Eggs were picked up and recorded each day, and the egg production percentage was calculated on a replicate basis. Every 2 weeks, feed intake (g/quail/day) was determined by weighing the remaining feeds from replicates. Also, all eggs collected from replicates were weighed separately to determine egg weight and averaged. Feed efficiency was calculated as consumed kg feed per kg egg and for a dozen eggs. Fifteen eggs from each main group (3 eggs from per replication group) were randomly taken to detect egg quality parameters every 2 weeks. Eggshell thickness was measured using an eggshell thickness gauge (Orka Technology Ltd, USA) from the top, middle and bottom parts of the eggshell after the shell membrane was removed. Eggshell breaking strength was detected with a cantilever system (5) and the values were recorded in Newton (N) unit. The heights of the albumen were measured in the egg samples, and then the haugh units belong to all groups were calculated by placing these values in the formula (13).

Measuring of chemical composition of the rosemary VO: The pure rosemary VO (Origin; Mersin /Turkey, Extraction Type; Hydro distillation, Density; 0.895 at 20 °C) used in this study were obtained from Semi Ethernic Oil Industry and Trade Limited Company. Gas chromatography analysis of rosemary VO was carried out on an MS-Thermo Polaris Q GC-Thermo Trace GC (Thermo Ficher inc, MA, USA) ultra-fitted with a fused HP5-MS capillary column (Thermo Ficher inc, MA, USA) (30 x 0.25 x film thickness 0.5 μ m). The temperature was programmed to rise from 95 °C to 240 °C at 4 °C /min. Samples were injected in split mode at 250 °C. Helium gas was used as the carrier at a pressure of 1.3610 atm.

Determination was made with FID (250 °C) and the injection volume was the same for all samples (8.1 µl). Chromatograms were detected using MS or MS/MS. Internal standards were taken into account in the calculation of data (43).

Thiobarbituric acid (TBA) analysis of egg yolk: At the end of this study, 45 egg yolk samples (15 from each main group) were measured for MDA levels. The lipid oxidation value of egg yolk samples stored at 4 °C in the refrigerator was determined on 1, 7 and 28 days. MDA levels in eggs from experimental groups were measured as a secondary oxidation product according to the TBA method which is a spectrophotometric method defined by Ke et al. (34). A modification of the 2-thiobarbituric acid method was used, and the results were 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 MDA.

Statistical analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences version 22.0 (SPSS, Chicago, IL, USA). One way ANOVA was used to evaluate the effects of rosemary VO on the performance parameters (egg weight, egg production, feed intake, feed efficiency), egg quality (shape index, eggshell thickness, eggshell breaking strength, haugh unit, yolk color) and egg oxidative stability (MDA levels). Values were expressed as arithmetic means \pm standard error of the mean (SEM). Tukey test was used as a post hoc test and the level of significance used in all of the tests was $P < 0.05$ (55).

Results

In the study, the analysed VO components of rosemary supplemented to the quail diets are shown in Table 1. As can be seen from the table, the main active ingredients of rosemary VO were 1,8-cineol (43.96 %), α -pinene (25.33 %), camphene (11.09 %) and 3-carene (10,7 %), respectively. Specific gravity value and levels of rosemary VO are summarised in Table 2. The specific gravity value of rosemary VO was 0.895 g/mL. The amounts of rosemary VO supplemented to the quail diets were calculated according to the specific gravity value of the VO. Ingredients and nutrient composition of the basal diet are given in Table 3. The analyzed dry matter, ether extract, ash, total phosphorus, calcium values and the calculated metabolizable energy value of the basal diet was within the normal range for laying quails reported by NRC (42). In this study, no significant difference was found on the feed intake, feed efficiency and egg weights among the control and experimental groups. The supplementation of rosemary VO at the levels of 200 and 400 mg/kg to the diets caused an increase in quails' egg production ($P < 0.01$). However, there was no effect on the

Table 1. Analysed chemical composition of rosemary VO.

<i>Rosemary VO</i>	
<i>Components</i>	<i>(%)</i>
1,8-cineole (Eucalyptol)	43.96
α -Pinene	25.33
Camphene	11.09
3-carene	10.70
D-limonene	2.5
Limonene	1.77
Ocimen	1.68
β -pinene	1.40
Camphor	0.73
Bornyl acetate	0.04
Borneol	0.03
Isoborneol	0.02
Caryophyllene	0.02

Table 2. Specific gravity value and amounts of rosemary VO supplemented to quail diets.

Specific Gravity*	Control	Group1	Group2
0.895 g/mL	-	200 mg/kg	400 mg/kg
	-	0.22 ml	0.44 ml

*The amounts of rosemary VO supplemented to the treatment diets were calculated using the specific gravity value of VO.

Table 3. Ingredients and nutrient composition of the basal diet (as fed basis).

<i>Ingredients, %</i>	
Corn	45.00
Soybean Meal	23.15
Full Fat Soybean Meal	13.00
Wheat	4.00
Sunflower Meal	3.00
Vegetable Oil	3.80
Calcium Carbonate	6.40
Dicalcium Phosphate	1.00
NaCl	0.30
DL-Methionine	0.10
Vitamin-Mineral Premix ^a	0.25
<i>Analyzed values, %</i>	
Metabolisable Energy, Kcal/kg ^b	2919.69
Dry matter	89.00
Crude Protein	20.60
Ether Extract	7.94
Ash	10.84
Calcium	2.53
Total Phosphorus	0.60
Available Phosphorus ^c	3.55

^a Provides per kg diet: α -tocopherol acetate 20 mg, retinol 2.4 mg, cholecalciferol 0.075 mg, riboflavin 3 mg, thiamin 3 mg, pyridoxal 3.5 mg, niacin 20 mg, cyanocobalamin 0.01mg, folic acid 1mg, biotin 0.03 mg, pantothenic acid 4mg, choline 600 mg, Fe 60 mg, Cu 5 mg, Zn 60 mg, Mn 80 mg, I 1, Se 0.15 mg, Co 0.2 mg..

^b Carpenter and Clegg equation (35) was used to calculate the ME level.

^c Calculated value.

Table 4. Effects of rosemary VO supplementation on the performance parameters¹ of laying quails from 1 to 56 days of age.

Rosemary VO (mg/kg)	Control		Group 1		Group 2		P
	0		200		400		
Feed intake, g/d	34.31	± 1.04	36.96	± 1.94	37.63	± 0.17	NS
Egg production, %	73.47	± 2.60 ^b	83.98	± 1.96 ^a	82.19	± 1.93 ^a	<0.01
Egg weight, g	11.00	± 0.10	11.11	± 0.09	11.60	± 0.10	NS
Feed efficiency, kg feed/kg egg	4.35	± 0.21	4.05	± 0.14	4.02	± 0.13	NS
Feed efficiency, kg feed/ a dozen eggs	0.58	± 0.03	0.54	± 0.02	0.56	± 0.01	NS

^{a, b}: Different letters within the same row indicate significant differences among the groups.

NS: Not significant ($P > 0.05$).

¹Egg weight refers to the average of all eggs collected from 5 replicates. Values of egg production, feed intake and feed efficiency measured on the basis of group are means of 5 replicates, $n = 5$.

exterior (shape index, eggshell thickness, breaking strength) and interior (haugh unit, egg yolk colour) egg quality parameters. No difference was determined in the 1st day egg yolk MDA values of the eggs obtained from the control and treatment groups and stored in a refrigerator at +4 °C. However, a significant decrease in MDA levels in egg yolk was observed on the 7th ($P < 0.001$) and 28th ($P < 0.01$) days in storage with both doses of rosemary VO.

Discussion and Conclusion

The main active phenolic compounds of rosemary VO supplemented to quail feeds in this study were 1,8-cineol (43.96 %), α -pinene (25.33 %), camphene (11.09 %) and 3-carene (10,7 %), respectively. Barakat and Ghazal (6) and, Santana-Méridas et al. (50) stated that carnosic acid, carnosol, camphene, caffeic acid, ursolic acid, rosmarinic acid, 1,8-cineole, α pinene are the most active antioxidant components found in rosemary VO. However, the VO composition of aromatic herbs may vary depending on the seasons, soil structure and climate factors.

In this study, dietary rosemary VO significantly increased egg production of laying quails (83.98 and 82.19 vs 73.47, $P < 0.01$), but did not affect egg weight, feed intake and feed efficiency parameters (Table 4). There are contradictory results about the effect of dietary herbal extracts on performance parameters of broiler, laying hen and quails. While some researchers reported that herbal extracts positively affect performance parameters such as body weight gain, egg production, feed intake and feed efficiency (7, 27, 58), the others stated that they had no significant effect on these parameters (11, 32, 37, 52). Yesilbag et al. (59) investigated the effects of rosemary VO (200 mg/kg), oregano VO (200 mg/kg) and their mixtures (40 mg/kg rosemary VO + 160 mg/kg oregano VO) on performance and egg quality parameters in laying quails. The authors reported that there was no significant

difference between the groups in body weight and egg weight. However, they determined that the rosemary VO significantly improved egg production and feed efficiency, while the mixtures of volatile oils caused a significant increase in feed intake. Simsek et al. (53) investigated the effects of dietary rosemary VO (200 mg/kg), cinnamon VO (200 mg/kg) and a mixture of these volatile oils (100 mg/kg rosemary VO + 100 mg/kg cinnamon VO) on quails. At the end of the study, there was no difference between the groups in feed intake. While the highest egg weight was obtained from the rosemary VO group, the highest egg production and feed efficiency belonged to the cinnamon VO group. In a study conducted by Hernandez et al. (29) to determine the effect of two herbal extract mixtures (5000 mg/kg sage + thyme + rosemary and 200 mg/kg oregano + cinnamon + pepper) on broiler performance, a little performance enhancer effect of herbal extract mixtures as in this study was seen, but none of the supplementations caused significant effects. Jamroz and Kamel (30), and Jang et al. (31) reported that herbal extracts or their volatile oils caused an increase in broiler performance by stimulating the secretion of digestive enzymes and increasing the digestibility of nutrients in feed. It has been suggested that the inconsistency in the effectiveness of herbal extracts on the performance of different poultry species may depend on many factors such as the composition of the basal diet, feed intake and environmental conditions or harvesting time of the herbs, extraction methods, conservation conditions and the differences in the levels of extracts supplemented to the diets (12, 16, 28, 40).

No significant effect of rosemary essential oil on eggshell breaking strength, eggshell thickness, shape index, haugh unit and egg yolk colour were determined in groups fed diets containing rosemary VO at levels of 200 mg/kg and 400 mg/kg (Table 5). Similarly, rosemary VO supplementation to the laying hen diets did not have any significant effects on shape index, eggshell weight,

eggshell thickness, eggshell breaking strength, haugh unit and yolk colour (9, 23, 47). On the other hand, Cufadar et al. (17) stated that the eggshell breaking strength and eggshell thickness significantly increased in laying hens fed with 250 mg/kg rosemary VO supplemented diet when compared to the control group. Alagawany and Abd El-Hack (2) also demonstrated that performance and egg quality parameters were improved by supplementation of 3000 mg/kg rosemary powder to laying hen diets. The number of studies examining the effects of rosemary VO on egg quality parameters in quails is limited. In a study conducted on laying quails, it was determined that rosemary VO significantly increased the egg white and yolk index values and affected the egg yolk colour positively (59). In a word, the supplementation of rosemary VO at different levels to the laying quail diets in the current study did not significantly affect the egg quality parameters.

The effect of rosemary VO on lipid oxidation of egg yolk stored in the refrigerator (+4 °C) for 1, 7 and 28 days was determined by measuring the MDA level which is secondary oxidation product according to the TBA method. As the degree of oxidation in yolk lipids increases during eggs storage, the level of MDA measured in egg yolk also increases. There was no difference in the 1st day egg yolk MDA (TBA-reagent) values of the eggs obtained from control and treatment groups and stored in a refrigerator at +4 °C in the present study. However, a significant decrease was determined in MDA levels of egg yolk in both levels of rosemary VO (200 and 400 mg/kg diet) on the 7 (0.05 and 0.07 vs 0.44, $P < 0.001$) and 28

(2.97 and 3.03 vs 6.59, $P < 0.01$) days of storage (Table 6). Our data are in agreement with previous results reported on the antioxidant effect of rosemary VO. Radwan et al. (47) propounded that the supplementation of 1 % rosemary VO to the diet of hens significantly reduced MDA values in eggs stored at room temperature compared to the control diet. Lopez-Bote et al. (38) and Tang et al. (56) also found that the dietary rosemary VO had the potential to increase the antioxidant capacity of chicken meat. It is well known that major phenolic compounds, carnosic acid, carnosol, camphene, caffeic acid, ursolic acid, rosmarinic acid, 1,8-cineole, α pinene and other phenolic compounds are the most active antioxidant components found in rosemary VO (3, 6, 50). Galobart et al. (25) reported that there was a delay in iron-induced lipid oxidation in hen eggs fed on diets containing rosemary extract, and carnosic acid in rosemary could act as an effective antioxidant when included in the diet at levels of 500 mg/kg or above. Krause and Ternes (36) established that if the hen diets contain 500 or 1000 mg/kg of carnosic acid, the rate of carnosic acid transfer from diet to egg is 0.0025 %. It was determined that the rosemary VO used in the present study contains 1,8-cineol (43.96 %), α -pinene (25.33 %), camphene (11.09 %) and 3 carene (10.7 %) (Table 1), among the main phenolic compounds in rosemary mentioned above. In this study, the significant decrease in MDA levels of egg yolk stored in the refrigerator can be explained by the fact that phenolic hydroxyl groups in rosemary VO delay the formation of hydroxyl peroxide by giving hydrogen to peroxide radicals at the beginning of lipid oxidation (19, 20).

Table 5. Effects of rosemary VO supplementation on egg quality parameters¹ of laying quails.

Rosemary VO (mg/Kg)	Control		Group 1		Group 2		P
	0		200		400		
Shape index	77.28	± 0.29	77.72	± 0.24	76.22	± 0.56	NS
Eggshell thickness, μ m	0.13	± 0.00	0.14	± 0.00	0.14	± 0.00	NS
Eggshell breaking strength, N/cm ²	13.17	± 0.23	13.05	± 0.24	13.11	± 0.29	NS
Haugh unit	90.62	± 0.54	90.12	± 0.60	89.77	± 0.53	NS
Yolk color	11.72	± 0.06	11.65	± 0.06	11.80	± 0.06	NS

NS: Not significant ($P > 0.05$).

¹Values are means of 15 eggs (3 eggs per replicates), n=15.

Table 6. Effects of rosemary VO supplementation on MDA levels¹ (mg MDA/kg sample) in egg yolk samples at different storage time (+4 °C).

Rosemary VO (mg/kg)	Control		Group 1		Group 2		P
	0		200		400		
Day 1	0.05	± 0.01	0.04	± 0.01	0.03	± 0.01	NS
Day 7	0.44	± 0.10 ^a	0.05	± 0.01 ^b	0.07	± 0.01 ^b	<0.001
Day 28	6.59	± 0.52 ^a	2.97	± 0.52 ^b	3.03	± 1.00 ^b	<0.01

^{a, b}: Different letters within the same row indicate significant differences among the groups.

NS: Not significant ($P > 0.05$).

¹Values are means of 15 eggs (3 eggs per replicates), n=15.

In conclusion, supplementation of 200 mg/kg and 400 mg/kg of rosemary VO to the laying quail diets increased egg production without adversely affecting other performance parameters and egg quality, and reduced egg yolk MDA values during storage in the refrigerator. Based on the data obtained from this research and the information provided from the literature, it has been concluded that rosemary VO can be used as a natural antioxidant in laying quail diets to extend eggs shelf life and to minimize quality losses during storage, considering its strong antioxidant effect.

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Ethical Statement

This study was approved by the Uludag University Animal Experiments Local Ethics Committee (Decision no. 2018-15/04).

Conflict of Interest

The authors are declared that there is no conflict of interest.

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