Effects of dietary fennel volatile oil on performance, egg quality, and egg yolk oxidative stability of laying quails

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ABSTRACT

This study was conducted to determine the effects of adding different levels of fennel volatile oil (VO) to the laying quail diets on performance, internal-external egg quality parameters, and eggs's oxidative stability. A total of 105 (6-week-old) laying quails (*Coturnix coturnix* Pharaoh) were used, and the quails were separated into 3 treatment groups, including 5 replicated sub-groups. A basal diet not containing fennel VO was formulated for the control group, and fennel VO was added to the basal diet at the levels of 200 and 400 mg/kg for Group 1 and Group 2, respectively. Diets and water were provided to quails ad libitum. Treatments continued for 56 days. As a result, the addition of fennel VO to the quail diets did not affect feed efficiency, haugh unit, egg yolk color, egg weight, shape index, or egg shell breaking strength. However, both levels of fennel VO increased feed intake ($P<0.05$), egg production ($P\leq0.001$), eggshell thickness ($P<0.001$), and the rate of damaged eggs ($P<0.05$). Although there is no difference in malondialdehyde (MDA) levels of egg yolks stored at +4 °C on day 1, both levels of fennel VO significantly reduced MDA levels of egg yolks on days 7 and 28 ($P<0.01$). The fact that fennel VO could be added to poultry rations as a natural feed additive to increase performance, improve eggshell quality, and minimize storage losses by extending the egg shelf life was concluded based on the data obtained.

Introduction

As is well known, maintaining a healthy diet is possible by consuming an adequate amount of animal protein on a daily basis. In this context, poultry products have significant value in meeting the animal protein demand of the increasing world population due to their health benefits and cost-effectiveness. For many years, antibiotic growth promoters were used in poultry nutrition to increase yield and product quality. However, the haphazard use of these agents has reached a level that poses a threat to public health, leading to residues in poultry meat and eggs. Thus, in the early 2000s, the use of growth-promoting antibiotics in poultry diets was banned in many developed countries. After growth-promoting antibiotics were banned, natural feed additives (prebiotics, probiotics, aromatic plants, and the volatile oils extracted from those aromatic plants) became one of the core concepts in the poultry industry. Scientific attention has been drawn to the demonstrated effects of herbal extracts, including antimicrobial (4, 17, 31, 39), antiviral (4, 7), antiparasitic (38), anti-inflammatory (4), antifungal (4, 28), antioxidant (4, 8, 27, 40), immunostimulatory (4), hypolipidemic (44), palatability increaser (48), and digestion improver (10, 18, 26), as indicated by numerous studies on poultry.

Fennel (*Foeniculum vulgare* Mill), an aromatic herb belonging to the Apiaceae family, has been used in medicine or to add flavor to foods since ancient times (33, 42). The active components that provide flavor and aroma to the VO of fennel seeds are anethole, fenchone, estragol,

1,8-cineole, para-cymene, β-myrcene, linalool, alpha-pinene, beta-pinene, γ-terpinene, camphene, camphor, and 3-methylbutanol (15). The main active component of fennel VO is trans-anethole, a phenolic ester (34). The amount of anethole consists of 60–70% of the VO extracted from fennel seeds, depending on which part of the plant it is obtained from (19, 24).

Recently, the issue of maintaining healthy nutrition has occupied the agenda, which has led to a remarkable increase in the demand for foods containing natural preservatives by people all around the world. In this regard, studies are conducted by nutritionists in order to detect the protective effects of natural feed additives, especially aromatic plants and their VOs, and increase the quality and shelf life of poultry products. These studies have mostly focused on rosemary, thyme, turmeric, cloves, and the VOs obtained from these aromatic plants, which have strong antibacterial and/or antioxidant capacities. There are a limited number of studies investigating the effects of fennel on poultry compared to the aforementioned aromatic herbs. In addition, these studies focusing on fennel use mainly focused on broiler chickens (1, 18, 32, 47), and a limited number of studies have been conducted on layer quails. Moreover, there are very few studies that determine the chemical composition of fennel essential oil and also correlate the results by taking into account the existing active components contained in VO. It has been proven that the VO obtained from different parts of the fennel herb, especially its seeds, has antimicrobial (24), antioxidant, and hepatoprotective (40, 43) effects.

Our hypothesis was that fennel VO added to laying quail diets may extend the egg shelf life via its antioxidant characteristics and increase performance due to its antimicrobial effect by improving intestinal health. It was also thought that herbal VO may improve the performance of quails due to their general effects, including increasing feed palatability, promoting feed intake, and increasing the digestive juices and enzyme activity by enhancing liver functions. The aim of this study is to detect the effects of different levels of fennel VO added to the diet of laying quails on performance, internal-external egg quality parameters, and eggs's oxidative stability.

Material and Methods

Animal material and management: This study was conducted under the confirmation of the Bursa Uludağ University Ethics Committee (decision number: 2018-15/05). A total of 105 (6-week-old) laying quails (*Coturnix coturnix* Pharaoh) were used, and the quails were separated into 3 treatment groups based on their live body weight. In addition, the main groups were separated into 5 replicated sub-groups, each of which consists of 7 quails. Quails were placed in cages with an area of 112 cm^2 for each of them. Diets and water were provided ad libitum. The research was continued for 56 days. Quails were subjected to 18 hours of light and 6 hours of darkness per day. During the research, it was taken care to keep the henhouse temperature at 23°C and the humidity at 55–60%.

Experimental diets and analyses: Quails in the treatment groups were fed with an isocaloric and isonitrogenic basal diet based on corn and soybean. In the formulation of the basal diet, reference values reported by NRC (36) were taken into account to meet the requirements of laying quails. No antioxidants were added to the diet other than the basic level of vitamin E included in the premix (Table 3). In the research, a basal diet that did not contain fennel VO was prepared for the control group, and fennel VO was added to the basal diet at 200 and 400 mg/kg levels for Group 1 and Group 2, respectively. To prevent losses in the effectiveness of fennel VO, feeds were prepared weekly and then immediately placed in ziplock bags. Additionally, feed bags for each replicate were placed in buckets with lids, and feeding was done twice a day. After fennel VO was obtained from a commercial company, its chemical composition was analyzed by gas chromatography-mass spectrometry (GC-MS). Fennel VO levels to be added to the laying quail diets were determined based on the literature (11, 14, 49) and the recommendations of the supplier company. The specific gravity value of fennel VO required to calculate the amount of fennel VO to be added to the trial diets was obtained from the supplier company. AOAC procedures were followed to determine the crude nutrient composition of the basal diet (5). Calcium (20) and total phosphorus (22) levels were determined using spectrophotometric methods. The basal diet's metabolizable energy level was calculated by substituting the crude nutrient values obtained from the analyses into the equation developed by Carpenter and Clegg (13).

Performance parameters: Eggs were collected and recorded daily, and the number of damaged eggs among groups in the replicate basis was also noted. Egg production percentages were calculated on a replicate-group basis. The amount of feed given to the groups was recorded, and the remaining feed from the replicate groups was weighed every 2 weeks to determine the daily feed intake of the quails. Additionally, to determine the egg weights of the groups, all eggs taken from the replicate groups were weighed one by one, and the average egg weight was calculated. The feed efficiency of quails in groups was expressed as kg of feed consumed for the production of one kg and one dozen eggs.

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Internal and external egg quality parameters: In order to determine the internal and external egg quality parameters of the groups, a total of 45 eggs were collected randomly every 2 weeks: 15 eggs from each treatment group (3 eggs from each repetition) for each measurement (4 x 45 = 180 eggs during the research period). Egg shell thickness was determined with an egg shell thickness gauge (Orka Technology Ltd., USA). After the shell membranes were removed, measurements were made on the shell samples taken from the upper, middle, and lower parts of the eggs, and their averages were taken. Eggshell breaking strength was determined in Newton units using a console system (6). The egg yolk color was determined with a Roche yolk color scale from 15 (dark orange) to 1 (light pale). In addition, the thick albumen height in the egg samples was measured, and the haugh unit of the eggs was calculated by taking into account the weight value of the same egg (12).

Determination of the chemical composition of fennel VO: Pure fennel VO, obtained by the hydro distillation method and originating from Mersin, Türkiye, was used. The chemical composition of fennel VO was analyzed on an MS-Thermo Polaris Q GC-Thermo Trace GC (Thermo Ficher Inc., MA, USA) ultra-fitted with a fused HP5 MS capillary column. The column temperature was programmed to increase by 4 °C per minute from 95 °C to 240 °C. 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 performed by FID (250 °C), and the injection volume was 8.1 µl for all samples. MS or MS/MS was used to determine chromatograms, and data were calculated using internal standards (37).

TBA Analysis: At the end of the treatments, the level of MDA, a secondary oxidation product, was measured in 15 egg yolk samples from each treatment group (3 eggs from each replicated sub-group) in order to detect the oxidative stability of the eggs. Egg yolk samples were stored in the refrigerator at +4 °C, and the yolk lipid oxidation value was determined spectrophotometrically by the TBA analysis method (30) on the 1st, 7th, and 28th days.

Statistical Analysis: Statistical Package for Social Sciences version 22.0 (SPSS, Chicago, IL, USA) was used to statistically evaluate the research data. Performance parameters (feed intake, feed efficiency, egg production, egg weight), internal-external egg quality parameters, and yolk MDA level were evaluated with a one-way ANOVA test. Values are expressed as the arithmetic mean ± standard error of the mean (SEM). The Tukey test was used as a post hoc test, and the significance level was considered as $P<0.05$ in all tests applied to the research data (45).

Results

The chemical composition, specific gravity value (0.961 g/mL), and amounts of fennel VO added to the diets of laying quails are given in Tables 1 and 2, respectively. The specific gravity value of fennel VO was used to calculate the amount of VO to be added to quail diets. The main active components of fennel VO were trans-anethole (70.58%), fenchone (10.50%), estragole (5.01), 1,8-cineole (3.20%), γ-terpinene (2.03%), p-cymene (1.32%), β-myrcene (1.30%), and linalool (1.30%). The ingredients and chemical composition of the basal diet are shown in Table 3. The nutrients (dry matter, ash, crude protein, ether extract, calcium, and total phosphorus) and calculated metabolizable energy value of the basal diet detected by analysis were found within the normal range reported by the NRC for laying quails (36).

The addition of different levels of fennel VO to laying quail diets did not affect feed efficiency or egg weight. However, both levels of fennel VO increased feed intake ($P<0.05$) and egg production ($P\leq0.001$). The internal (haugh unit, egg yolk) and some external (shape index, eggshell breaking strength) quality parameters of eggs were not affected by the addition of fennel VO to the diets. At both levels of fennel VO, eggshell thickness increased significantly ($P<0.001$), and a decrease in the rate of damaged eggs was observed ($P<0.05$). While no difference was detected in the MDA levels of egg yolks stored in the refrigerator (+4 °C) among the control and experimental groups on day 1, both levels of fennel VO reduced egg yolk MDA levels on days 7 and 28 ($P<0.01$).

Table 1. Analysed Chemical Composition of Fennel VO.

Fennel VO	
Components	**(%)**
Trans-Anethole	70.58
Estragole	5.01
Fenchone	10.50
1,8-cineole	3.20
γ-Terpinene	2.30
p-cymene	1.32
Linalool	1.30
β-myrcene	1.30
α-Pinene	0.59
Camphor	0.40
β-pinene	0.30
3-methylbutanol	0.20

Table 2. Specific Gravity Value and Amounts of Fennel VO Supplemented to Quail Diets.

Specific Gravity*	**Control**	**Group 1**	**Group 2**
0.961 *g/mL*	-	*200 mg/kg diet*	*400 mg/kg diet*
	-	0.208 *ml*	0.416 *ml*

*This value was used to calculate the amount of fennel VO to be added to treatment diets.

Table 3. Basal Diet's Ingredients and Chemical Composition (as fed basis).

Ingredients, %	
Corn Grain	45.19
Soybean Meal (CP, 44%)	22.95
Full Fat Soybean (CP, 36%)	13.00
Wheat	4.00
Sunflower Meal (CP, 28%)	3.00
Vegetable Oil	3.70
$CaCO_3$	6.41
DCP	1.00
NaCl	0.30
DL-Methionine	0.10
Vitamin-Mineral Premix[a]	0.25
Analyzed values, %	
Dry matter	89.85
Crude Protein	20.40
Ether Extract	7.88
Ash	10.72
Calcium	2.57
Total Phosphorus	0.60
Available Phosphorus[b]	0.35
Metabolisable Energy, Kcal/kg[b]	2902.69

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

Discussion and Conclusion

In this study, the main phenolic compounds in fennel VO added to laying quail diets were trans-anethole, fenchone, estragole, 1,8-cineole, γ-terpinene, linalool, β-myrcene, and p-cymene (Table 1). The composition of VO in aromatic herbs can be affected by many factors, like the type and variety of the herb, soil structure, climate, harvest time, extraction method, and storage conditions of the extract.

The addition of fennel VO to the laying quail diets in the experimental group significantly increased feed intake compared to the control group (Group 1: 37.47 and Group 2: 36.93 vs. Control: 34.37) (Table 4). While both dietary levels of fennel VO significantly increased egg production in quails, it did not affect feed efficiency (kilograms of feed: kilograms of eggs, kilograms of feed: dozen eggs). Buğdaycı et al. (11) added fennel seeds to the diets of laying quails at levels of 0.3%, 0.6%, and 0.9%, and they reported that fennel had no effect on feed intake and feed efficiency (kilogram feed: dozen eggs) parameters. Nasiroleslami and Torki (35) investigated the effects of adding 300 mg/kg ginger or 300 mg/kg fennel VO to the diets on laying hens's performance. In the study, neither ginger nor fennel VO affected feed intake, feed efficiency, or egg production. Contrary to the studies mentioned above, there are studies that support the performance data obtained from the current study as well. In a study conducted by Yeşilbağ (49), the 300 mg/kg level of fennel VO in the diet caused a significant increase in feed efficiency and egg production in laying quails. In another study (2), the effects of phytogenic herbs on performance and egg quality in laying hens were examined, and fennel seeds, black cumin seeds, and hot red pepper were added to the diets at the same level (5 g/kg). As a result, it was stated that the best results were obtained from quails fed with a fennel seed-supplemented diet. Kazami-Fard et al. (29) reported that fennel VO at a level of 50 mg/kg in the diet significantly increased the egg production of broiler chicken breeders. Sachdev et al. (41) suggested that the increase in egg production in poultry may be due to the presence of unsaturated fatty acids necessary for egg production, such as linolenic acid in fennel extract. It is stated that a significant percentage of the fatty acids contained in fennel seeds are linoleic (71.31%) and linolenic (11.66%) fatty acids (3). In this study, it is considered that the higher feed intake of quails in the groups fed with diets containing fennel VO contributed to an increase in egg production.

Table 4. Performance Parameters of Laying Quails.

	Control	**Group 1**	**Group 2**	
Fennel VO (mg/kg)	**0**	**200**	**400**	**P**
Feed intake, g/d	34.37 ± 0.67 [b]	37.47 ± 0.47 [a]	36.93 ± 1.17 [a]	<0.05
Egg production, %	73.32 ± 2.58 [b]	83.32 ± 1.12 [a]	82.24 ± 1.77[a]	≤0.001
Egg weight, g	11.00 ± 0.10	11.07 ± 0.11	11.15 ± 0.12	>0.05
Feed efficiency, kg/kg	4.35 ± 0.21	4.08 ± 0.08	4.01 ± 0.11	>0.05
Feed efficiency, kg/dozen	0.58 ± 0.03	0.54 ± 0.01	0.54 ± 0.02	>0.05

The difference between the means with different letters in the same row is significant (P<0.05).

Table 5. Egg Quality Parameters of Laying Quails.

	Control	Group 1	Group 2	
Fennel VO (mg/kg)	**0**	**200**	**400**	**P**
Shape index	77.28 ± 0.29	77.90 ± 0.38	77.22 ± 0.55	>0.05
Eggshell thickness, µm	0.13 ± 0.00[b]	0.15 ± 0.00[a]	0.15 ± 0.00[a]	<0.001
Eggshell breaking strength, N/cm^2	13.17 ± 0.23	13.00 ± 0.25	13.47 ± 0.25	>0.05
Haugh unit	90.43 ± 0.49	90.44 ± 0.54	90.25 ± 0.50	>0.05
Yolk color	11.72 ± 0.06	11.67 ± 0.07	11.73 ± 0.06	>0.05
Damaged egg, %	1.05 ± 0.26[a]	0.55 ± 0.14[b]	0.39 ± 0.14[b]	<0.05

The difference between the means with different letters in the same row is significant (P<0.05).

Table 6. MDA Levels in Egg Yolk Samples.

	Control	Group 1	Group 2	
Fennel VO (mg/kg)	**0**	**200**	**400**	**P**
Day 1	0.052 ± 0.010	0.036 ± 0.007	0.038 ± 0.007	>0.05
Day 7	0.438 ± 0.100[a]	0.136 ± 0.022[b]	0.105 ± 0.009[b]	<0.01
Day 28	6.794 ± 0.408[a]	5.489 ± 0.177[b]	5.102 ± 0.216[b]	<0.01

The difference between the means with different letters in the same row is significant (P<0.05).

The addition of fennel VO to the laying quail diets did not have any effect on the shape index, breaking strength, haugh unit, or yolk color of the eggs in the current study. However, both levels of fennel VO resulted in an increase in eggshell thickness and a decrease in the rate of damaged eggs (Table 5). Bugdaycı et al. (11) added fennel seeds at different levels (0.3%, 0.6%, and 0.9%) to the diets of laying quails, and the researchers stated that fennel did not cause any effect on the internal and external quality parameters of the eggs. In another study (35), the addition of 300 mg/kg ginger or 300 mg/kg fennel VO to laying hen diets had no effect on egg quality parameters except haugh unit and eggshell thickness. It was observed that hens fed with a diet containing 300 mg/kg fennel VO had lower haugh units but higher eggshell thickness compared to the other groups. Yeşilbag (49) reported that rosemary (300 mg/kg) or fennel (300 mg/kg) VO added to the diets of laying quails improved eggshell thickness, yolk color, and haugh unit. In the study, it was expressed that there was no significant difference in egg weight, egg mass, egg shape index, or egg breaking strength between the control and experimental groups. Moreover, Gharaghani et al. (23) reported that the increase in damaged egg rate, which occurs as a result of low feed intake and poor egg calcification in laying hens raised under heat stress, can be greatly alleviated by adding fennel to the diets. It is stated that this may be related to the decrease in the amount of oxidative products formed in the reproductive organs due to the presence of antioxidant components in fennel and its positive effect on eggshell calcification.

Briefly, in this study, adding fennel VO to quail diets had no effect on egg internal and external quality parameters except shell thickness and damaged egg rate. In this study, the improvement in eggshell quality of quails in the experimental groups may be due to the effect of anethole, which is the main active component of fennel VO. In our study, the analyzed anethol level of fennel VO added to the quail diets was 70.58% (Table 1). It has been propounded that anethole has an estrogenic effect similar to phytoestrols and that steroidal estrogens increase the intestinal absorption of calcium by activating the 1-α-hydroxylase enzyme in the kidneys (46). Additionally, in the current study, the decreased rate of damaged eggs in groups fed diets containing fennel VO may be related to higher calcium intake as a result of fennel VO increasing feed intake.

MDA levels in the yolks of eggs kept in the refrigerator (+4 ºC) on the 1st, 7th, and 28th days of storage were determined according to the TBA analysis procedure. The purpose of this analysis was to determine the effect of fennel VO on lipid oxidation. In parallel with the increase in the oxidation degree of lipids in the egg yolk during storage, there is an increase in the level of MDA, which is the secondary oxidation product. Although there is no significant difference in egg yolk MDA values on the first day of storage, egg yolk MDA levels were decreased on the 7th (0.136 and 0.105 vs. 0.438) and 28th (5.489 and 5.102 vs. 6.794) days of storage in both levels of fennel VO (200 or 400 mg/kg diet).

The data obtained are consistent with the results of previous studies reporting that fennel VO reduces the level

of MDA by preventing the oxidation of lipids in the egg yolk. In a study by Cengiz (14), 50:50% rosemary or fennel (100 mg/kg rosemary + 100 mg/kg fennel or 200 mg/kg rosemary + 200 mg/kg fennel) VO was added to quail diets, and diets enriched with rosemary and fennel VO contributed a significant decrease in meat MDA level on the 15th day of storage at +4 °C compared to the control group. Deniz et al. (16) stated that 200 or 400 mg/kg rosemary VO levels in laying quail diets remarkably reduced egg yolk MDA values on the 7th and 28th days of storage at +4°C. 1,8-cineole and α-pinene, which are among the most active antioxidant components in rosemary VO (27), are among the active components of fennel VO used in this research. Gharaghani et al. (23) reported that an increase in egg MDA levels was detected in laying hens exposed to heat stress, whereas MDA levels decreased significantly in eggs obtained from groups fed diets containing different levels of fennel fruits (0, 10, and 20 g/kg). In the current study, it is thought that the decrease in MDA levels in the egg yolks of the experimental groups is due to other antioxidant active components (1,8-cineole, α-pinene, etc.), especially the anethole in fennel VO.

Based on the literature mentioned above, it is noteworthy that the studies investigating the effects of herbal extracts on poultry have different results (performance parameters, product quality, etc.), although the same poultry species and herb extract are used. It is suggested that this may be due to many factors, including the composition of the basal diet and extract level used, extraction methods, feed intake, and the variability in environmental conditions (9, 25).

Consequently, the addition of 200 or 400 mg/kg fennel VO to laying quail diets increased feed intake, egg production, and eggshell thickness. Meanwhile, both levels of fennel VO reduced the egg yolk MDA values during storage of eggs at +4 °C and damaged egg rate without adversely affecting other parameters of the studies. Based on the data obtained from laying quails, it was concluded that fennel VO could be used as a natural feed additive in poultry diets to increase performance, improve eggshell quality, and minimize storage losses by extending the egg shelf life. Additionally, the results of this study proved that fennel VO is a beneficial source as a natural antioxidant.

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Ethical Statement

This study was carried out after the animal experiment was approved by Bursa Uludağ University Local Ethics Committee (Decision No: 2018-15/05).

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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