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Research Article

Effect of Peppermint Oil (*Mentha piperita*) Supplementation in Quail Diets on Egg Production and Some Egg Quality Characteristics, Oxidative Stress Parameters and Lipid Profile

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Abstract

This study investigated the effects of peppermint oil supplementation in the diet of Japanese quail on egg production, quality characteristics, oxidative stress parameters, and lipid profile. A total of 90 female quail were divided into three groups: a control group, a low-dose group receiving 150 mg/kg peppermint oil, and a high-dose group receiving 300 mg/kg supplementation. The study lasted for 8 weeks during which external and internal egg quality characteristics, biochemical and lipid profile analyses were performed. The results indicated that low doses of peppermint oil had beneficial effects on eggshell weight and quality. In contrast, high doses of peppermint oil had negative effects on egg internal quality unit (IQU) and increased egg pH. A significant increase in omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), was observed in the high-dose peppermint oil group, indicating an improvement in the lipid profile. While the low dose of peppermint oil improved certain egg quality parameters, the high dose had negative effects, highlighting the importance of determining the correct dosage of supplementation. Overall, the study shows that peppermint oil, when used at the appropriate dose, can improve both egg quality and lipid profiles, potentially offering health benefits by increasing beneficial fatty acids such as omega-3 in quail eggs.

Keywords: Egg quality, Lipid profile, Oxidative stress, Peppermint, Quail

Introduction

The growing global demand for poultry products has led to a significant increase in production, particularly of eggs, which are now one of the most widely consumed sources of animal protein. This surge in consumption can be attributed to factors such as easy accessibility, affordability and broad cultural acceptance. In addition to chicken eggs, quail eggs have become a popular alternative due to their nutritional benefits and ease of production. As poultry production intensifies, there is growing interest in using natural feed additives and plant-based products to improve animal health and productivity in response to dietary regulations, consumer preferences and animal welfare concerns [1-5].

With increasing restrictions on the use of antibiotics in animal agriculture, there has been a growing shift towards the use of botanical extracts as natural alternatives. These plant-derived compounds are used not only to prevent disease, but also to promote growth, enhance development and improve overall performance in poultry and livestock [6,7]. Among these, polyphenols-micronutrients found in abundance in plant extracts-have attracted attention for their diverse biological activities. They are known for their cardiovascular, cognitive, antioxidant, anti-inflammatory and immunomodulatory properties, among others [1]. However, despite these benefits, excessive levels of polyphenols may have uncertain or even negative effects on gastrointestinal health, nutrient digestion, enzyme

activity, vitamin and mineral absorption, and ultimately on laying performance and egg quality [6-8].

Peppermint is an aromatic plant that is widespread throughout the world and includes 25 different species. It can grow in all climatic conditions. Peppermint is widely used in medicine as an antiseptic, antibacterial, antispasmodic, emetic and diuretic due to its essential oils (menthol, menthyl acetate, menthone, mentofuran, carvone) [2,9,10]. Herbal extracts have also been reported to have a beneficial effect on biochemical parameters, particularly by lowering blood cholesterol levels [3,4,10-12]. The use of caraway seeds and black cumin seeds in the diets of chickens also reduced the levels of total cholesterol (TCHOL) and low-density lipoprotein cholesterol (LDL-CHOL) in the blood serum of broiler chickens [13,14]. It has been reported that sage and thyme extracts from aromatic plants have positive effects on some performance and quality parameters, can be used as lipid oxidation inhibitors instead of vitamin E, which is an antioxidant, and can be added to laying hen diets as cholesterol lowering agents [15]. Studies with peppermint oil and peppermint extract show the potential of peppermint as an additive [16]. Among the herbal extracts, the addition of chamomile, wild mint and thyme extracts had no significant effect on egg production, egg weight, feed intake, feed conversion ratio, egg quality characteristics and hatching performance [17]. In another study, peppermint oil was found to significantly decrease total oxidative status (TOS) and increase total antioxidant status (TAS) in blood [18]. They showed that the addition of lavender essential oil to the diet resulted in an increase in live weight, egg production, egg mass, egg weight, egg white height, Haugh unit (HU), omega-3 fatty acids and a decrease in malondialdehyde (MDA) concentration [19]. In the studies, the use of herbal extracts in different forms and ratios did not show significant effects on egg quality, but did show significant effects on egg biochemical profiles.

Considering the effects of different herbal extracts on different poultry species and products, the aim of this study was to investigate the effects of peppermint on egg quality parameters, biochemical properties and lipid profile. The main objective of the study was to investigate the effects of different levels (low and high doses) of peppermint oil in quail diets on eggs.

Material and Methods

Ethical Approval

This study was conducted with the permission of Kırıkkale University Animal Experiments Local Ethics Committee dated 19.07.2023 and numbered 2023/07/28.

Animal Material

The study utilized a total of 90 female Japanese quail *(Coturnix coturnix Japonica)* with an age of 16 days. The animals were weighed to ensure that there was no difference in live weight among all groups and then divided into three experimental groups with three replications, each with 10 quails. Each subgroup was housed in cages measuring 100x45x20 cm. The study continued for 8 weeks after the first eggs were observed at 16th day and were terminated at the conclusion of the period. Feed and water were provided *ad libitum*, and a comfortable temperature (24°C) was maintained for all animals throughout the study. Additionally, a 16-h light and 8-h dark cycle was applied during the laying period.

Diet Content

The animals utilized in the experiment were provided with the dietary regimen recommended by the National Research Council (NRC) [20], tailored to the specific age groups from hatching until the conclusion of the experiment, with consideration given to the basal diet. From the time of hatching until the 16th day, when the first egg was seen, the broiler chick feed was used and then the experiment was started by switching to the layer feed. The nutrient contents of the diets were determined according to the methods specified in AOAC [21]. Metabolizable energy of the diets was calculated according to TSE [22]. The nutritional values of the basal diet used during the pre-laying and laying periods have been determined *(Table 1)*. The experimental groups were structured as follows: Group 1, basal diet (control), Group 2, basal diet + 150 mg/kg peppermint essential oil (LM), and Group 3, basal diet + 300 mg/kg peppermint essential oil acid (HM).

Determining Egg Production and Egg Quality

In the study, the number of eggs collected daily was determined, and the number of eggs per week was calculated by including those with broken, cracked, and shell anomalies. For the 8-week experimental period, the groups were evaluated in terms of egg numbers. Fifteen eggs were randomly collected from each group at the beginning, middle and end of the experiment and egg weight (EW), egg shape index (ESI) and shell surface area (ESSA) were calculated. The formula of Yannakopoulos and Tserveni-Gousi [23] was used to calculate the egg shape index.

Table 1. *Basal diets nutritient values*

Nutrient (%)	Broiler Chicken	Laying Hen
Dry matter	88.61	90.41
Crude Protein	22.19	16.88
Crude fat	5.05	4.17
Crude Cellulose	4.69	5.54
Crude Ash	5.48	6.33
ME, Kcal/Kg	3093	2701

Egg shape index (%) = (Egg width/Egg length) x 100

The formula of Olawumi and Chiristiana [24] was used to calculate the eggshell surface area.

Shell surface area (cm^2) = 3.9782 x (Egg weight) $^{0.7056}$

After the measurements and calculations of external quality characteristics were completed, internal quality characteristics were evaluated. The examined characteristics were determined as membrane shell weight (ESW), eggshell ratio (ESR), albumen height (AH), albumen width (AW), albumen length (AL), yolk height (YH), yolk width (YW), yolk length (YL), albumen index (AI), yolk index (YI), albumen pH value, Haugh Unit (HU) and Internal Quality Unit (IQU). The pH of the egg yolk was determined using a digital pH meter (HANNA, HI 2221). The formulas given below were used in the calculations.

Shell ratio (%) = (Shell weight (g)/Egg weight (g)) x 100 [25]

Albumen index (%) = (Albumen height (mm)/[(Albumen length + Albumen width)/2] x 100 [24].

Yolk index (%) = (Yolk height (mm)/Yolk diameter (mm)) x 100 [23].

Haugh Unit = 100 x log (Egg height (mm) + 7.52 – 1.7 x Egg weight (g) $^{0.37}$) [26].

IQU (%) = 100 x log [Egg height (mm) + 4.18 – (0.8989 x Egg weight (g) $^{0.6674}$)] [25].

Determining the Amount of Cholesterol in Egg Yolk

One day before the end of the experiment and on the day the experiment was to be terminated, three eggs from each quail group were collected and the cholesterol and triglyceride content of the egg yolk was determined by Uyanık et al.[27] with slight modification. The egg yolk was separated from the egg white, and then the yolk was collected from the vitelline membrane with a syringe. Weigh out 0.1 g of egg yolk and place it in a homogenizing tube. Add 1 mL of 99.7% isopropyl alcohol. The raw egg yolk was vortexed until dissolved and homogenized. The homogenate in the tube was incubated at 37°C for 10 min and centrifuged at 3000 rpm for 5 min at 4°C. Egg yolk cholesterol (CHO) concentration was determined in supernatant samples using a commercial kit (Otto Scientific, Türkiye) in a autoanalyzer (Mindray BS-400, China).

Determination of TOS and TAS Levels in Egg Yolk

One day before the end of the experiment and on the day the experiment was to be terminated, three eggs from each quail group were collected and the TAS and TOS levels of the egg yolk were determined according to the method reported by Kornbrust et al.[28] with modification. 1 mL of egg yolk was taken by piercing the vitelline membrane with an automatic pipette tip, and Raw egg yolk was diluted 1:3 with distilled water. The mixture in the tube was vortexed until a completely homogeneous solution was obtained. The tubes were centrifuged at 15000 rpm for 5 min at +4°C. After centrifugation, TAS and TOS values in supernatant were determined using commercial test kits (Rel Assay, Türkiye) on an autoanalyzer device (Mindray BS-400, China). To calculate the OSI, which is known as the percentage of the ratio of total oxidative capacity to total antioxidative capacity, the units of TAS and TOS levels were equalized. The results were expressed in arbitrary units (AU) [29]. Oxidative stress index (OSI) was calculated using the following equation:

$$OSI = \frac{TOS\ (\mu mol\ H2O2\ Equiv/L)}{TAS(\mu mol\ \ Trolox\ Equiv/L)}$$

Determination of Egg Lipid Profile

Egg yolk samples were obtained from freshly collected eggs at the conclusion of the study (eight eggs from each group, for a total of 24 eggs). The fatty acids (free and bound) in the egg yolk samples were subjected to a modified three-step methylation procedure, as described by Wang et al.[30]. The supernatants, comprising methylated fatty acids in n-hexane, were transferred to a 1.5-mL screw-neck ND-9 amber vial with 9-mm screw caps (silicone white/PTFE) and analyzed using a gas chromatograph (TRACE 1300, Thermo Scientific, USA) with automatic sampling (Thermo AI 1310, Thermo Scientific, USA). The FAME mix (37°C) standard solution in dichloromethane (Chem-Lab, CL.40.13093.0001, Zedelgem, Belgium) was employed for the identification of the peaks. Heptadecanoic acid (C17:0) was employed as the internal standard. The processing method employed was that of a fatty acid methyl esters column (length 60 m, internal diameter 0.25 mm, film thickness 0.25 µm, maximum temperature 250-260°C), with an injection split temperature of 255°C, a column temperature of 140°C, and a flow rate of 30 mL/min. This was conducted for a period of 42 min. The identification of the fatty acids was conducted by comparing the peaks in the chromatogram with the standard retention times [31].

Statistical Analysis

In this study, the full factorial model was employed with the use of IBM SPSS 27 [32] in order to analyze a number of egg quality parameters across three experimental groups: the control group, the low-dose peppermint oil group (with a dose of 150 mg/kg), and the high-dose peppermint oil group (with a dose of 300 mg/kg). The inclusion of interaction terms was not considered appropriate given the simplicity of the experimental design. A generalized linear model (GLM) was selected as the analytical method

for the comparison of continuous dependent variables (egg quality characteristics, biochemical parameters and lipid profiles) across categorical independent variables (treatment groups). This approach was deemed appropriate due to the experimental design, thereby facilitating a robust comparison of means between groups. Polynomial contrasts (linear, quadratic, cubic) were not applied in this analysis, as the primary objective was to compare the main effects of the treatment groups on the dependent variables without exploring trend analyses. The parameters subjected to analysis included: The external egg quality characteristics included in the analysis were shell weight and shell thickness. The internal egg quality characteristics were yolk weight, albumen weight, egg internal quality unit (IQU) and egg pH. The biochemical and lipid profile parameters were omega-3 fatty acids (EPA and DHA). A full factorial GLM model was employed to conduct statistical comparisons between the three experimental groups. After the GLM procedure, Tukey's multiple comparison test was used to separate the statistically different groups (P<0.001).

Results

In the study, the effects of adding different amounts of peppermint oil to the diet on egg production, and external egg quality parameters were investigated *(Table 2)*.

Upon analysis of the egg numbers, it was observed that the control group exhibited the highest average egg number, while the HM group demonstrated the lowest. Statistically significant differences were identified between the groups in regard to egg numbers (P<0.001). In terms of EW values, a significantly higher value was observed in the LM group compared to the control group, while this value was significantly lower in the HM group (P=0.014). No statistically significant difference was observed between the groups in terms of ESI (P=0.431). The ESW was found to be significantly higher in the HM group compared to the control and LM groups (P=0.003). Although no statistically significant difference was observed between the groups in terms of ESR, a marginally higher value was noted in the HM group (P=0.128). The ESSA values were found to be similar in the LM and control groups, but significantly lower in the HM group (P=0.005).

Table 2. *Egg external quality parameters in groups containing different amounts of peppermint oil (LSM±S_E)*

Parameters	Control	LM	HM	P
EN	136.87±2.33^{c}	125.87±1.27^{b}	114.12±1.27^{a}	<0.001
EW	9.89±0.10^{ab}	10.22±0.09^{b}	9.35±0.31^{a}	0.014
ESI	80.35±0.30	79.53±0.37	79.55±0.74	0.431
ESW	1.32±0.01^{a}	1.31±0.02^{a}	1.44±0.03^{b}	0.003
ESR	13.50±0.22	12.30±0.17	15.55±1.95	0.128
ESSA	19.78±0.13^{b}	19.72±0.11^{b}	18.40±0.54^{a}	0.005

EN: Egg number; EW: Egg weight, g; ESI: Egg shape index, %; ESW: Egg shell weight, g; ESR: Egg shell ratio, %; ESSA: Egg shell surface area. LM: Low mint group; HM: High Mint group.
^{a,b,c} *different letters in the same line represent statistical difference (P<0.01)*

Table 3. *Internal egg quality parameters in groups containing different amounts of peppermint oil (LSM±S_E)*

Parameters	Control	LM	HM	P
AH	4.57±0.11	4.61±0.08	4.54±0.12	0.907
YH	10.64±0.29	10.89±0.16	9.89±0.47	0.095
AW	33.36±0.54	33.83±0.35	32.91±0.38	0.327
AL	43.11±0.53^{ab}	44.71±0.39^{b}	40.67±1.24^{a}	0.003
YW	24.77±0.52^{b}	23.60±0.16^{a}	23.62±0.23^{ab}	0.026
pH	8.78±0.04^{a}	8.68±0.05^{a}	9.28±0.03^{b}	<0.001
YI	44.31±1.90	47.17±0.62	44.14±1.80	0.306
AI	3.02±0.11^{a}	2.89±0.05^{a}	6.34±0.83^{b}	<0.001
HU	94.47±0.99^{b}	90.92±0.29^{a}	88.23±1.03^{a}	<0.001
IQU	70.25±0.69^{c}	66.06±0.71^{b}	60.05±1.82^{a}	<0.001

AH: Albumen height, mm; YH: Yolk height, mm; AW: Albumen width, mm; AL: Albumen length, mm; YW: Yolk width, mm; YI: Yolk index, %; AI: Albumen index, %; HU: Haugh Unit; IQU: Internal quality unit
^{a,b,c} *different letters in the same line represent statistical difference (P<0.01)*

Table 4. *Effects of dietary peppermint oil supplementation on oxidative stress parameters (LSM±S_E)*				
Parameters	**Control**	**LM**	**HM**	**P**
TAS	0.210±0.02	0.350±0.07	0.199±0.06	0.159
TOS	6.30±1.45	7.97±4.17	8.18±1.39	0.856
OSI	3.78±1.37	6.28±4.45	6.03±1.32	0.777
TG	457.90±9.53	459.36±4.34	442.71±7.59	0.237
CHOL	29.29±1.81	34.66±3.04	31.07±2.39	0.336

*TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, TG: Triglyceride, CHOL: Cholesterole. LM: Low mint group; HM: High mint gro*up

Table 5. *Egg lipid profile in groups containing different amounts of peppermint oil (LSM±SE)*				
Fatty Acids	**Control**	**LM**	**HM**	**P**
Lauric Acid Methyl Ester (C12:0) 4% (dodecanoate)	0.010±0.001	0.021±0.001	0.018±0.001	0.324
Tridecanoic Acid Methyl Ester (C13;0) 2%	0.005±0.001	0.006±0.002	0.002±0.001	0.450
Myristic Acid Methyl Ester (C14:0) 4%	0.246±0.025	0.260±0.021	0.240±0.020	0.817
Myristoleic Acid Methyl Ester (C14:1) 2%	0.015±0.008	0.010±0.000	0.008±0.001	0.531
Pentadecanoic Acid Methyl Ester (C15:0) 2%	0.046±0.007	0.057±0.005	0.046±0.004	0.356
cis-10-Pentadecenoic Acid Methyl Ester (C15:1) 2%	0.023±0.001	0.029±0.001	0.024±0.002	0.075
Palmitic Acid Methyl Ester (C16:0) 6%	20.029±1.233	23.658±1.568	20.614±1.436	0.177
Palmitoleic Acid Methyl Ester (C16:1) 2%	2.504±0.199	2.770±0.218	2.415±0.262	0.530
Heptadecanoic Acid Methyl Ester (C17:0) 2%	0.073±0.007$^{a.b}$	0.093±0.009^{b}	0.046±0.012^{a}	0.011
cis-10-Heptadecenoic Acid Methyl Ester (C17:1) 2%	0.036±0.001	0.040±0.003	0.040±0.004	0.702
Stearic Acid Methyl Ester (C18:0) 4%	6.190±0.365^{a}	8.445±0.656^{b}	6.552±0.724$^{a.b}$	0.033
Elaidic Acid Methyl Ester (C18:1n9t) 2%	0.027±0.011	0.003±0.002	0.008±0.004	0.062
Oleic Acid Methyl Ester (C18:1n9c) 4%)	30.969±1.063	37.018±2.172	36.641±2.462	0.078
Linolelaidic Acid Methyl Ester (C18:2n6t) 2%	26.165±3.638	11.490±5.223	19.388±5.436	0.124
Linoleic Acid Methyl Ester (C18:2n6c)2%	10.150±1.048	11.535±0.719	10.041±1.270	0.535
γ-Linolenic Acid Methyl Ester (C18:3n6) 2% (GLA)	1.775±0.081^{a}	2.245±0.104^{b}	2.031±0.147^{ab}	0.029
Arachidic Acid Methyl Ester (C20:0) 4% (Eicosanoic acid)	0.060±0.003	0.081±0.006	0.079±0.007	0.043
cis-11-Eicoenioic Acid Methyl Ester (C20:1) 2% (gondoic acid)	0.128±0.014	0.136±0.016	0.070±0.023	0.045
α-Linolenic Acid Methyl Ester (C18:3n3) 2% (ALA)	0.090±0.012	0.085±0.002	0.093±0.007	0.827
Heneicosanoic Acid Methyl Ester (C21:0) 4%	0.010±0.000^{a}	0.020±0.002^{b}	0.024±0.003^{b}	0.002
cis-11,14,17-Eicosadienoic Acid Methyl Ester (C20:2) 2%r	0.039±0.002	0.052±0.004	0.044±0.004	0.078
Behenic Acid Methyl Ester (C22:0) 4%	0.046±0.001	0.073±0.017	0.033±0.010	0.080
cis-8,11,14-Eicosatrienoic Acid Methyl Ester (C20:3n6) 2% (DGLA)	0.012±0.001	0.017±0.001	0.013±0.001	0.066
Erucic Acid Methyl Ester (C22;1n9) 2%	0.000±0.000	0.000±0.000	0.000±0.000	-
cis-11,14,17-Eicosatrienoic Acid Methyl Ester (C20:3n3) 2% (ETE)	0.100±0.011	0.145±0.017	0.124±0.007	0.071

Table 5. *Egg lipid profile in groups containing different amounts of peppermint oil (LSM±SE) (continued)*

Fatty Acids	Control	LM	HM	P
Arachidonic Acid Methyl Ester (C20:4n6) 2% (AA)	0.315±0.024	0.431±0.030	0.373±0.044	0.075
Tricosanoic Acid Methyl Ester (C23:0) 2%	0.055±0.002	0.074±0.005	0.066±0.008	0.120
cis-13,16-Docosadienoic Acid Methyl Ester (C22:2) 2%	0.290±0.020	0.306±0.037	0.171±0.071	0.122
Lignoceric Acid Methyl Ester (c24;0) 4% (Tetracosanoate)	0.241±0.026	0.255±0.033	0.274±0.031	0.756
cis-5,8, 11,14,17-Eicosapentaenoic Acid Methyl Ester (c20:5n3) 2% (EPA)	0.114±0.016[a]	0.200±0.013[b]	0.235±0.030[b]	0.002
Nervonic Acid Methyl Ester (C24:1) 2% (cis-15-tetracosenoate)	0.048±0.005	0.065±0.007	0.051±0.009	0.270
cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3) (DHA)	0.178±0.033	0.331±0.016	0.241±0.074	0.099
Saturated Fatty acids, %	28.726±1.575	35.206±2.265	29.946±1.927	0.064
Unsaturated Fatty acids	71.261±1.579	64.745±2.245	70.056±1.926	0.061
MUFA	33.749±1.225	40.070±2.318	39.256±2.652	0.102
PUFA	37.513±2.783	24.675±4.543	30.800±4.368	0.098
ω-3	0.481±0.042[a]	0.761±0.039[b]	0.693±0.069[b]	0.003
ω -6	37.031±2.761	29.914±4.578	30.107±4.411	0.091
ω -9	31.139±1.087	37.166±2.185	36.726±2.462	0.082
ω -3/ω -6	0.013±0.001[a]	0.042±0.007[b]	0.031±0.008[a.b]	0.017

MUFA: Mono-unsaturated fatty acids; PUFA: Polyunsaturated fatty acids; ω-3, 6, 9: Omega 3, 6, 9. LM: Low mint group; HM: High mint group
[a,b] different letters in the same line represent statistical difference (P<0.01)

In this study, the effects of adding different amounts of peppermint oil to the diet on internal egg quality parameters in quails were investigated *(Table 3)*.

No statistically significant difference was observed between the groups in terms of albumen height (P=0.907). The egg yolk height was observed to be higher in the LM group; however, no statistically significant difference was identified (P=0.095). No statistically significant differences were observed between the groups in terms of albumen width and yolk width (P=0.327 and P=0.026). However, the yolk width was found to be numerically lower in the LM group. The lowest albumen length was observed in the HM group while the highest albumen length was observed in the LM group. This difference was statistically significant (P=0.003). With regard to yolk width, a significantly higher value was observed in the control group (P=0.026). The egg pH value was found to be significantly higher in the HM group, with a highly statistically significant difference (P<0.001). Upon examination of the albumen index, a significant increase was observed in the high peppermint oil group, which was found to be statistically highly significant (P<0.001). The Haugh Unit and the Internal Quality Unit were observed to be diminished in the HM group. Both parameters demonstrated statistically significant discrepancies (P<0.001).

In the study, the effects of adding different amounts of peppermint oil to the diet on egg yolk cholesterol and oxidative stress levels were investigated *(Table 4)*.

The present study examined specific biochemical and oxidative stress parameters in the control group, LM, and HM groups. The TAS values were observed to be higher in the LM group in comparison to the control group. However, TAS values in the HM group was found to be comparable to that of the control group. Nevertheless, no statistically significant difference was identified between the groups (P=0.159). With regard to TOS, the HM and LM groups exhibited higher values than the control group. However, these differences were not statistically significant (P=0.856). The oxidative stress index (OSI) was observed to be higher in the LM and HM groups in comparison to the control group. Nevertheless, this discrepancy was not statistically significant (P=0.777).

With regard to lipid parameters, TG levels were found to be in close proximity to those observed in the control group in the LM group and exhibited a slight decline in the HM group. However, these differences were not statistically

significant (P=0.237). The CHOL values were observed to be higher in the LM group than in the control group, and similar in the HM group. However, these differences were not statistically significant (P=0.336).

In the study, the effect of adding different amounts of peppermint oil to the diet on egg lipid profile was investigated *(Table 5)*.

As a result of the analysis, some differences were detected between the groups in terms of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). In particular, a significant increase was observed in the HM group compared to the control and LM groups in terms of omega-3 fatty acids (ω-3) (P<0.05). EPA and DHA levels were found to be significantly higher in the HM group. The HM group also exhibited a higher value compared to the other groups in terms of omega-3/omega-6 ratio (P<0.05). In addition, SFA concentrations were relatively higher in the control group, no statistically significant differences were detected between the groups (P>0.05). Some saturated fatty acids such as Stearic Acid (C18:0) and Palmitic Acid (C16:0) were observed to be at lower levels in the HM group. On the other hand, MUFA were observed at the highest levels in the HM group, but this difference was not found to be statistically significant (P>0.05). Although there were no statistically significant differences between the groups in terms of PUFA. PUFA levels were found to be higher in the HM group than in the LM group.

Discussion

In recent years, there has been a notable increase in research investigating the effects of peppermint oil on egg production, egg weight, shell quality, and other quality parameters. A substantial body of research has demonstrated the pronounced impact of peppermint oil and other essential oils on the productivity and egg quality of hens. It has been demonstrated that the addition of EOM to the diet of laying hens has a beneficial impact on egg-laying performance [33]. Upon examination of the results pertaining to egg number, the findings diverged from those of previous studies. These previous studies had indicated that the incorporation of herbal extracts into poultry diets did not result in a notable alteration in egg number [34]. The existing literature on the effect of aromatic plants and essential oils on egg quality presents a range of findings. A study by Çabuk et al. [35] reported that a 24 mg/kg EO mixture supplementation increased egg production, feed efficiency and decreased the rate of broken eggs. The same study reported that essential oil mixtures demonstrated favorable effects on egg production rate and feed conversion ratio (FCR) in quails. In a study conducted by Büyükkılıç Beyzi et al.[36], no significant difference was observed between the EOS and control groups with respect to egg quality parameters. However, a notable increase in egg specific gravity was evident in the group that received the essential oil and vitamin mixture during the final four weeks of the study period. It was observed that LM group resulted in an increase in egg weight. The significantly higher egg weight (10.22 g) observed in the LM group compared to the control group lends further support to this finding. It is also noteworthy that this value exhibited a marked decline in the high peppermint oil group (9.35 g). This suggests that while the use of essential oils at appropriate doses may have beneficial effects, excessive doses may result in adverse outcomes. This finding is consistent with previous studies indicating that optimal doses of essential oils can enhance egg quality [33,34]. Additionally, other findings related to the use of essential oils indicate differences in eggshell characteristics. The eggshell weight was found to be significantly higher in the HM group than in the control and LM groups, thereby confirming the effects of essential oils on shell quality. Similarly, although no statistically significant difference was observed in shell ratio, a slightly higher value was noted in the high peppermint oil group. However, this increase did not reach statistical significance, which requires further investigation. However, it should be noted that high doses may have negative effects on egg internal quality and shell surface area. In order to confirm these effects of peppermint oil, longer term studies covering different dose levels are required.

The results on the effects of diets supplemented with peppermint oil on internal quality parameters of quail eggs are consistent with the literature on the effects of essential oils on egg quality. Most studies indicate that essential oils do not always have a direct effect on albumin quality [17,19,36]. Deniz et al.[37], reported that different essential oils can cause some changes in yolk volume and color, but these effects usually depend on the type and dose of oil. Florou-Paneri et al.[33], reported that some essential oils, such as peppermint oil, may affect egg pH and thus egg storage time. In this study, Eisen and Bohren [25] identified albumin level and Haugh unit as important indicators of egg freshness and stated that essential oils may cause differences in these indicators. Studies such as Frankič-Korošec et al.[38], also emphasize that essential oils should be used with caution and report that high doses may have negative effects on egg quality. In this study, no significant difference was observed between the groups with respect to albumin level (P=0.907). The increase in yolk height observed in the LM group is noteworthy but not statistically significant (P=0.095). In terms of albumin length, the lowest value was observed in the HM group and the highest value was observed in the LM group (P=0.003), supporting the dose-dependent effect of

peppermint oil. However, a higher value for yolk width was observed in the control group (P=0.026), which is consistent with the findings that yolk components may differ. In this study, a significant increase in egg pH was observed in the HM group (P<0.001), suggesting that essential oils may affect pH and thus egg internal quality. The increase in albumin index and decrease in Haugh unit (P<0.001) indicate the importance of a balanced use of essential oils that affect different aspects of egg quality. These results are consistent with the literature showing that peppermint oil may contribute positively to quail egg quality at appropriate doses but may be detrimental at high doses.

Menthol and menthone, the major components of peppermint oil, have the ability to enhance antioxidant defenses by neutralizing free radicals and increasing cellular antioxidant activity [2]. Several previous studies have shown that moderate doses of essential oils, especially peppermint oil, potentiate endogenous antioxidant systems by increasing TAS in animals [9]. Previous studies have reported that high doses of peppermint oil may lead to lipid peroxidation, which may increase TOS levels [10]. Orzuna-Orzuna et al.[39], reported that essential oils may have pro-oxidant effects at high concentrations in poultry diets, which may lead to increased OSI levels. The non-significant differences in TAS, TOS and OSI levels between groups demonstrate the complex dose-dependent nature of the effects of peppermint oil on oxidative stress. The results of this study are consistent with broader research highlighting the importance of determining the optimal dose to maximize the antioxidant benefits of essential oils while avoiding adverse pro-oxidant effects. In the present study, oxidative stress parameters including TAS, TOS and OSI were found to be higher in the LM group than in the control group, but the difference did not reach statistical significance (P=0.159). This finding is consistent with the literature on the antioxidant properties of peppermint oil [10]. On the other hand, TOS levels were higher in both LM and HM groups compared to the control group, but these differences were not statistically significant (P=0.856). This suggests that while peppermint oil activates antioxidant pathways at low doses, it may trigger oxidative reactions at high doses. OSI levels followed a similar pattern and were higher in the LM and HM groups compared to the control group (P=0.777). Although not statistically significant, this trend indicates a balance between increased antioxidant capacity and oxidative stress.

The effects on the lipid parameters obtained in this study are in agreement with the existing literature and the research supports the possible effects of essential oil on lipid metabolism. Mehri et al.[40] and Barbalho et al.[41], reported that the effects of peppermint oil on serum lipid profile and cholesterol synthesis usually occur at low and moderate doses, but this effect depends on the type and dosage. Similarly, Arjun et al.[42], investigated the effects of peppermint oil on performance and lipid metabolism in poultry and reported positive results. The fact that TG levels in the LM group were close to the control group and slightly decreased in the HM group, but not statistically significant (P=0.237), is consistent with previous studies showing that peppermint oil may have a limited effect on lipid metabolism. The fact that CHOL levels were higher in the LM group compared to the control group and similar levels were observed in the HM group (P=0.336) is consistent with the findings that peppermint oil may affect cholesterol metabolism in different ways. These findings support the potential effects of essential oils in regulating lipid metabolism.

Studies suggest that peppermint oil may affect the biosynthesis and metabolism of polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids (ω-3), due to its bioactive compounds [19]. Some studies have also highlighted the potential impact of peppermint oil on the synthesis and degradation of saturated fatty acids (SFAs) like stearic acid (C18:0) and palmitic acid (C16:0), as well as its role in modulating monounsaturated fatty acids (MUFAs) such as oleic acid [36]. However, the results regarding the effect of peppermint oil on lipid metabolism remain inconsistent in the literature [10]. In this study, the effects of adding different amounts of peppermint oil to the diet on the lipid profile of eggs were investigated. The most notable finding was the significant increase in ω-3 fatty acid levels in the HM group compared to the control and LM groups (P<0.05). This is consistent with previous studies suggesting that essential oils may enhance ω-3 fatty acid metabolism [43]. Additionally, the ω-3/ω-6 ratio was higher in the HM group, supporting the hypothesis that peppermint oil may contribute to a more favorable lipid profile by modulating PUFA synthesis and storage processes. This finding is important as a high ω-3/ω-6 ratio is associated with health benefits, including anti-inflammatory effects and reduced cardiovascular risk [44]. While no significant differences in SFA concentrations were observed among the groups (P>0.05), the lower levels of specific SFAs (C18:0 and C16:0) in the HM group are noteworthy. These findings are in line with studies indicating that peppermint oil may influence the synthesis or degradation of SFAs [43]. The MUFA levels, although highest in the HM group, did not differ significantly among the groups (P>0.05). Given the health benefits of MUFAs like oleic acid, the observed increase in the HM group may still hold biological significance, potentially attributable to the stimulating effect of menthol on lipid metabolism [45]. For PUFA levels, while the differences between groups were not statistically significant, higher values were recorded in the HM group compared to the

LM group. This observation suggests a potential positive effect of peppermint oil on PUFA synthesis or storage, aligning with previous literature on the role of essential oils in modulating lipid metabolism [46].

In conclusion, the results of the study show that peppermint oil increases egg weight and shell quality at the correct dose, but this effect is reversed at high doses. Biochemically, high doses of peppermint oil (HM group) increased the levels of omega-3 fatty acids (EPA and DHA) and caused a significant increase in the omega-3/omega-6 ratio. When the lipid profile was analyzed, a decrease in saturated fatty acids, especially stearic acid and palmitic acid, was observed along with lower levels of saturated fatty acids (SFA). In addition, changes in MUFA and PUFA levels were observed, with higher PUFA levels in the HM group. In biochemical parameters related to the antioxidant defense system (TAS, TOS, and OSI), peppermint oil produced positive effects at low doses, while oxidative stress increased at high doses. These results suggest that peppermint oil may modulate lipid metabolism and contribute positively to egg quality; however, it is emphasized that the optimal dose of use should be carefully determined due to the negative effects of high doses.

Declarations

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author (Ş. Evci) on reasonable request.

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Ethical Approval

This study was conducted with the permission of Kırıkkale University Animal Experiments Local Ethics Committee dated 19.07.2023 and numbered 2023/07/28.

Declaration of Generative Artificial Intelligence: The authors declare that Generative Artificial Intelligence applications or softwares were not used in the study.

Conflict of Interest: The authors declared that there is no conflict of interest.

Author Contributions: ŞE: Study design, data collection, supervision, writing and editing; EE: Study design, data collection, statistical analysis, writing and editing; KK: Data evaluation, laboratory analysis; AŞ: Study design, data evaluation, laboratory analysis; MÇ: Study design, data evaluation, laboratory analysis.

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