**Original Article** Acta Vet Eurasia 2018; 44: 85-92

# The Effects of Drinking Water Supplemented with Essential Oils on Performance, Egg Quality and Egg Yolk Fatty Acid Composition in Laying Hens

Özlem KARADAĞOĞLU[1], Bülent ÖZSOY[2], Mükremin ÖLMEZ[3], Özlem DURNA AYDIN[3], Tarkan ŞAHIN[3]

[1]Department of Agricultural and Animal Production, Kars Vocational School, Kafkas University, Kars, 36100, Turkey

[2]Department of Animal Nutrition and Nutritional Disease, Faculty of Veterinary, Mustafa Kemal University, Hatay, 31000, Turkey

[3]Department of Animal Nutrition and Nutritional Disease, Faculty of Veterinary, Kafkas University, Kars, 36100, Turkey

**Cite this article as: Karadağoğlu, Ö., Özsoy, B., Ölmez, M., Durna Aydın, Ö., Şahin, T., 2018. The Effects of Drinking Water Supplemented with Essential Oils on Performance, Egg Quality and Egg Yolk Fatty Acid Composition in Laying Hens. Acta Vet Eurasia 44: 85-92.**

***ORCID IDs of the authors: Ö.K. 0000-0002-5917-9565; B.Ö. 0000-0003-0045-3790; M.Ö. 0000-0002-5003-3383; Ö.D.A. 0000-0003-4532-6795; T.Ş. 0000-0003-0155-2707***

## Abstract

This study was performed to examine the effect of different concentrations of an essential oil mixture (EOM) added to drinking water on growth performance, internal and external egg quality, and egg yolk fatty acid composition in laying hens. A total of 240 Brown Nick laying hens aged 20 weeks were used in this study. These hens were divided into one control and three treatment groups whose drinking water was supplemented with 0, 0.1, 0.2, and 0.3 ml/L of Oregofarm EOM. Oregofarm is a commercially available product containing peppermint, oregano, and anise oil. Each treatment group was further categorized into 10 subgroups, each consisting of 6 laying hens. The hens were fed basal rations for 16 weeks. For data collection and analysis, a 16-week period was divided into three time periods of 5 weeks. The weights of 25- to 30- and 35-week eggs were recorded weekly. In the groups whose drinking water was supplemented with the EOM, there was a significant improvement in egg production (EP) ($p<0.001$), weekly egg weights during the entire study period ($p<0.001$), and feed conversion ratio ($p<0.05$). Furthermore, egg shell thickness and yolk height were significantly increased ($p<0.05$) at week 35. The Haugh unit was also significantly improved ($p<0.05$) at weeks 25 and 30. However, feed intake and body weight were not affected by the treatment ($p>0.05$). The levels of saturated fatty acids were decreased, whereas the levels of polyunsaturated fatty acids increased with EOM supplementation, although the differences were not statistically significant ($p>0.05$). Therefore, based on the beneficial effects of supplementing drinking water with different concentrations of EOM on egg weight, egg production, feed conversion ratio, and egg yolk fatty acid compositions, it is suggested that EOM can be safely added at concentrations of up to 0.3 ml/L (i.e., the levels used in this study) to drinking water for laying hens.

**Keywords:** Egg quality, essential oils, laying hens, performance, yolk fatty acids

## Introduction

Due to cross and multiple resistance issues, many antibiotic growth promoters in poultry nutrition are restricted by the EU. Consequently, researchers have been exploring the use of phytogenic feed additives (PFA) such as plants, plant extracts, essential oils (EOs) and individual or combined active EOs ingredients (Bozkurt et al., 2014). Essential oils are a mixture of fragile and volatile compounds - often referred to as aromatic - by plant origin and plant rooting. Hence, the chemical composition and concentration of active plant compounds vary greatly dependent on their source (Hippenstiel et al., 2011). Mint (*Mentha piperita*) is a member of the Labiatae family and is probably of East Asian origin. Such herbal medicines are widely used and are believed to be particularly useful in combating the immune system and secondary infections (Akbari et al., 2015). As an aromatic plant growing mostly in the Mediterranean region, Oregano (*Origanum vulgare L.*) EO can be used as a phytogenic additive. The medicinal plant has been used as an anion for digestive, antiparasitic, antibacterial and antifungal stimulant effects (Ertaş et al., 2005).

**Address for Correspondence:** Özlem KARADAĞOĞLU • **E-mail:** drozlemkaya@hotmail.com

**Received Date:** 28 March 2018 • **Accepted Date:** 27 July 2018 • DOI: 10.26650/actavet.2018.410397

In some studies, the successful use of EOs as alternative growth promoters has already been proven and has started to play an important role in poultry nutrition. Phytogenic additives found in a wide variety of plants, spices and derivatives have a beneficial effect on the quality of animal products and animal health and are safe for use in the food industry (Ertaş et al., 2005). It has been reported that thyme EOs have various biological activities *in vitro* and *in vivo*, together with antimicrobial, antioxidant and antifungal effects (Espina et al., 2015; Mooyottu et al., 2014). Essential oils using herbs and spices might increase the acceptability of feed because of their flavorful characteristics and therefore, could advance feed intake by being added to poultry diets (Williams and Losa, 2001). Similarly, some studies showed positive effects on performance traits such as egg production rate, egg weight and egg mass output (Aydın et al., 2008; Bozkurt et al., 2012b), however some experiments showed that varying levels of dietary EOM did not significantly affect the performance, damaged eggs and eggshell weight (Olgun and Yıldız, 2014; Özek et al., 2011). There are limited studies reporting the effects of herbs and EOs on egg quality parameters of laying hens with contradictory results (Bölükbaşı et al., 2008 and 2010).

Due to increased dietary fat and increased coronary heart disease, egg yolk fatty acid composition is very important for consumers (Simopoulos and Salem, 1992). Methods of feeding animals can change the fatty acid composition of the eggs (Yi et al., 2014). Essential oils or mixtures added to diets have a positive effect on lipid metabolism (Acamovic and Brooker, 2005). Contrary to this statement, Ding et al. (2017) reported that there was no significant effect on the fatty acid composition of egg yolk in the experimental groups where Enviva EO additions were made and not made. Up to now, although the effects on the performance parameters of essential oil addition to poultry diets have been studied, there have very few reports on the effects of essential oils on egg yolk fatty acid composition. The study was designed to define the effects of essential oil mixture (EOM) (peppermint, oregano, and anise EOs) added to drinking water on growth performance, egg quality and egg yolk fatty acid compositions in laying hens.

## Materials and Methods

### Animals and experimental design

This study has been reviewed and approved by an ethical or advisory board of Animal Ethics Committee at Kafkas University (KAU-HADYEK/2016-032) (Kars, Turkey). A total of 240 *Brown-Nick* laying hens aged 20 weeks were used in the study. Drinking water was supplemented with EOs (Oregofarm EO, Farmavet International, Feed and Water Additives Specialist, Manisa, Turkey) at concentrations of 0.1, 0.2, and 0.3 mL/L respectively and were provided for 16 weeks (20 to 35 weeks). The EOM components are presented in Table 1. In the experiment, 4 groups of chickens were divided into 1 control group and 3 experimental groups. Ten sub-groups were created for each trial group and each sub group contained 6 laying chickens. In the study, the hens were fed with laying hen rations containing 16% HP and 2750 kcal / kg ME. The basal ration was prepared using maize and soybean meal. The ingredient and nutrient levels of the basal diet as presented in Table 2 met the NRC recommendations (1994).

The rations were applied to all groups as isocaloric and isonitrogenic mixes. Hens were sheltered in a hen house where light-

**Table 1.** The active componenets of the essential oil mixture

| | Components (mg/kg) |
|---|---|
| Thymol (thyme oil) | 2000 |
| B-phellanderene (thymeoil) | 1300 |
| Limonene (thyme oil+pepermint oil) | 3525 |
| B-pinene (thyme oil+pepermit oil) | 1977 |
| Linalool (oreganum oil) | 3645 |
| Carcacrol (oreganum oil) | 8910 |
| Anethole (anise oil) | 10712 |
| Menthole (pepermint oil) | 6375 |

**Table 2.** Composition and nutrient level of basal diet

| Ingredient composition | Basal Diet, $g/kg^{-1}$ |
|---|---|
| Corn | 620 |
| Soybean meal | 205 |
| Wheat bran | 61.8 |
| Oil | 10 |
| Limestone | 85 |
| Dicalcium phosphate | 12.9 |
| Salt | 2.5 |
| Vitamin mineral premix[a] | 2.5 |
| DL-Methionine | 0.3 |
| **Calculated nutrient levels** | |
| Metabolisable energy[b] (kcal $kg^{-1}$) | 2751 |
| Crude protein | 160 |
| Dry matter | 904 |
| Ether extract | 29.3 |
| Crude ash | 125 |
| Crude fibre | 28.4 |
| Calcium | 35.07 |
| Total phosphorus | 5.91 |
| Available phosphorus | 6.7 |
| Starch | 422.7 |
| Sugar | 33.4 |

[a]Supplied per kilogram of diet: 15372000 IU vitamin A, 6.28 mg vitamin E,0.64 mg vitamin K3, 37.36 mg Mn, 89 mg Fe, 25 mg Zn, 8.76 mg Cu, 0.03 mg Co, 0.05 mg MgI, 0.91 mg Se, 2400.000 IU $D_3$
[b]Metabolisable energy content of diets was estimated according to the equation of Carpenter and Clegg.

ing, temperature and ventilation were controlled. The temperature was maintained at approximately 22-24°C. The house had controlled lighting (16L: 8D). The experiment was completed in 16 weeks in the layer chicken unit of the Research and Application Farm at Kafkas University. All hens were supplied with diet and water *ad-libitum*. 15 liter water tanks were prepared for each trial group and connected via nipples. The daily water consumption of the animals was estimated. Fresh drinking water and EOM were added to the water tanks daily. The animals were constantly monitored and kept well hydrated throughout the experiment. The nutrient composition of the basal diet was determined according to the AOAC (1995).

**Performance and egg quality parameters**

In order to determine the changes in body weight, the body weights of the chickens were recorded at the beginning and end of the study. Egg production and weight and FI were registered weekly during the experiment. Egg production and FCR were calculated for each period. The magnitude of production was adjusted according to mortality rates, which were recorded daily. To determine egg quality parameters, in every 5 week period, 20 eggs were randomly selected per treatment (2 eggs per replicate). These were then evaluated in terms of eggshell weight, albumen and yolk weight, yolk height, eggshell thickness and HU. The eggs were weighed with a special instrument (TP-2000A-0.01 g, İstanbul, Turkey). The eggs were broken on a glass table. The eggshell thickness was measured at three different sections (upper-middle and ends) using a micrometer (Standardgage-200mm-8IN, Asia). The height of the yolk was measured with a tripod micrometer (Mitutoya-20mm, Kawasaki, Japan). Albumen quality was measured in terms of the HU calculated from the weight of the egg and the height of the albumen. While calculating HU values for each egg, the following formula was used:

$$HU = 100\log (H - 1.7W^{0.37} + 7.6)$$

(H is the observed height of the albumen (mm) and W is the weight of the egg (g)).

**Fatty acid profile of egg yolk**

During the final week of fatty acids analysis, the oil extraction was carried out on 10 egg samples from each group, according to the method laid out by the AOAC (1995), and then processed with methyl esters with Boron Trifluoride. The fatty acid methyl esters were condensed under nitrogen gas and then analyzed in GC-MS (HP 6890/5972). An Agilent HP88 100X250 micro-Mx250 mm column was used in the analysis. The initial temperature of the column was 120°C, while the final temperature was 230°C. The injector and detector temperature was set at 250°C. The injection speed was set at 50:1 and helium was used as a carrier gas.

**Statistical analysis**

The statistical analysis and the significance of the mean values between the groups were determined by the analysis of variance. The multiple range test was used to determine differences between the trial groups. The statistical analyses were performed with Statistical Package for the Social Sciences 16.0 (SPSS Inc.; Chicago, IL, USA) (Dawson and Trap, 2001).

## Results and Discussion

**Laying hen performance and egg production**

Initial and final BW, FI, FCR and egg production data for the layer hens fed drinking water containing EOM is presented in Table 3. Performance analysis showed that there were no effects on body weight and feed intake in the experimental group. During the experimental period, egg production and FCR improved significantly ($p<0.05$) with the supplementation of 0.2 and 0.3 mL/L EOM. Studies reported beneficial effects on laying performance with EOM supplementation in layer's diet (Bozkurt et al., 2012b; Özek et al., 2011). Radwan et al. (2008) reported that FI and BW were not significantly affected by dietary herbs. No differences in FI were reported in hens when the EO was supplemented to basal diets (Bozkurt et al., 2012a and 2012c; Çabuk et al., 2014). In contrast, Abdo et al. (2010) found that adding green tea EO in the ratio significantly reduced feed intake. Similarly, Bölükbaşı et al. (2008) found that EO supplementation in layer diets lowered FI and FCR was improved when the birds were fed EOM in their diet. Certain feeding experiments with layer hens didn't find any differences in FCR when the oregano EO was added to diets (Florou-Paneri et al., 2005). Similarly, Bozkurt et al. (2012a; 2012c) revealed that dietary inclusion of EO did not improve FCR in white layer hens. However, some beneficial effects on FCR using EO of inclusion level 24 mg/kg feed have been reported for the brown layer strain under hot environmental conditions (Çabuk et al., 2006). Some studies confirmed the positive effects of herbs and their respective EO on the BW of hens. Çabuk et al. (2006) reported that the BW of layer hens fed diets of an EO mixture increased compared to that of the control group over a 20-week period. Similarly, in another study, significant improvements were determined in the BW of hens when they were fed different levels of garlic (Khan et al., 2007). However, no effect was reported on BWG in response to dietary supplementation with tea leaves or green tea EO (Abdo et al., 2010), or dietary oregano EO (Florou-Paneri et al., 2005). Bozkurt et al. (2009) observed that adding an EO mixture to diet had no effect on egg production and weight. Similarly, Bölükbaşı et al. (2010) observed no effect on egg production but there was an increase in egg weight when diets were supplemented with thyme, sage or rosemary over a period of 12 weeks. However, Xianjiing He et al. (2017) found that added oregano EOs in layer diets significantly influenced egg production and average egg weight.

**Egg quality parameters**

The effects of Oregofarm EO on egg weight and internal and external egg quality are listed in Table 4 and 5. Supplementation of Oregofarm EO significantly increased the egg weight ($p<0.05$) weekly. In all periods, the egg weight was effected significantly

**Table 3.** The effects of adding EOM on the performance (mean±standard error) to layer hens' drinking water

| | Essential oil mixed supplementation (ml/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.1 | 0.2 | 0.3 | p |
| Initial body weight, g | 1734±17.63 | 1734±14.44 | 1771±16.55 | 1782±15.1 | 0.62 |
| Final body weight, g | 1736±62.91 | 1661±22.52 | 1742±21.99 | 1771±20.44 | 0.14 |
| Feed intake, g day$^{-1}$ per bird | 102.24±1.52 | 99.94±1.26 | 103.83±1.61 | 104.13±1.59 | 0.191 |
| Egg production, % | 90.88±0.44$^{b}$ | 89.74±0.48$^{b}$ | 92.41±0.41$^{a}$ | 92.90±0.40$^{a}$ | <0.001 |
| Feed conversion ratio, kg feed per kg egg | 2.01±0.04$^{a}$ | 1.97±0.03$^{ab}$ | 1.91±0.03$^{b}$ | 1.90±0.02$^{b}$ | 0.038 |

$^{a,b}$indicated the difference within a row was significant (p<0.05)

**Table 4.** The effects on the weekly egg weights of adding EOMs to layer hens' drinking water (g)

| | Essential oil mixed supplementation (mL/L) | | | | |
|---|---|---|---|---|---|
| Weeks | 0 | 0.1 | 0.2 | 0.3 | p |
| 1-wk | 55.00±0.59 (n:55) | 56.1±0.55 (n:53) | 56.36±0.64 (n:58) | 56.39±0.71 (n:59) | 0.36 |
| 2-wk | 55.66±0.55$^{b}$ (n:56) | 55.81±0.45$^{b}$ (n:56) | 57.48±0.55$^{a}$ (n:52) | 57.81±0.63$^{a}$ (n:55) | 0.007 |
| 3-wk | 56.8±0.64 (n:52) | 57.86±0.49 (n:54) | 58.47±0.64 (n:57) | 58.38±0.46 (n:55) | 0.15 |
| 4-wk | 56.7±.05 (n:59) | 57.07±0.45 (n:57) | 58.12±0.57 (n:55) | 58.25±0.59 (n:57) | 0.103 |
| 5-wk | 57.16±0.59$^{b}$ (n:58) | 56.89±0.43$^{b}$ (n:56) | 58.98±0.60$^{a}$ (n:59) | 58.40±0.52$^{ab}$ (n:57) | 0.019 |
| 6-wk | 57.17±0.52$^{b}$ (n:58) | 57.51±0.50$^{b}$ (n:59) | 58.17±0.61$^{ab}$ (n:57) | 59.18±0.52$^{a}$ (n:57) | 0.047 |
| 7-wk | 56.90±0.59$^{c}$ (n:48) | 57.56±0.47$^{bc}$ (n:52) | 59.62±0.65$^{a}$ (n:51) | 58.98±0.52$^{ab}$ (n:54) | 0.002 |
| 8-wk | 56.68±0.66$^{b}$ (n:55) | 56.93±0.56$^{b}$ (n:58) | 59.60±0.66$^{a}$ (n:58) | 59.33±0.58$^{a}$ (n:56) | 0.001 |
| 9-wk | 56.38±0.59$^{b}$ (n:48) | 56.35±0.57$^{b}$ (n:49) | 57.65±0.58$^{ab}$ (n:50) | 58.53±0.58$^{a}$ (n:53) | 0.019 |
| 10-wk | 57.44±0.70$^{b}$ (n:51) | 56.81±0.64$^{ab}$ (n:46) | 58.74±0.57$^{a}$ (n:55) | 59.13±0.61$^{a}$ (n:55) | 0.037 |
| 11-wk | 57.50±0.52$^{b}$ (n:47) | 58.92±0.61$^{ab}$ (n:49) | 59.77±0.63$^{a}$ (n:51) | 60.30±0.54$^{a}$ (n:57) | 0.005 |
| 12-wk | 57.75±0.67$^{c}$ (n:44) | 58.68±0.57$^{bc}$ (n:51) | 60.98±0.64$^{a}$ (n:53) | 60.39±0.59$^{ab}$ (n:53) | 0.001 |
| 13-wk | 57.61±0.72$^{b}$ (n:47) | 57.06±0.57$^{b}$ (n:47) | 60.25±0.52$^{a}$ (n:55) | 60.18±0.54$^{a}$ (n:56) | 0.000 |
| 14-wk | 57.87±0.73$^{b}$ (n:57) | 58.77±0.69$^{ab}$ (n:50) | 60.42±0.59$^{a}$ (n:55) | 59.80±0.50$^{a}$ (n:56) | 0.024 |
| 15-wk | 58.24±0.75$^{b}$ (n:51) | 57.58±0.54$^{b}$ (n:54) | 59.27±0.57$^{ab}$ (n:56) | 60.57±0.60$^{a}$ (n:56) | 0.003 |
| 16-wk | 57.55±0.69$^{b}$ (n:54) | 57.81±0.63$^{b}$ (n:55) | 60.37±0.69$^{a}$ (n:56) | 60.27±0.59$^{a}$ (n:56) | 0.001 |
| All Periods | 56.67±0.16$^{b}$ (n:840) | 57.34±0.14$^{b}$ (n:846) | 59.17±0.16$^{a}$ (n:878) | 59.11±0.15$^{a}$ (n:892) | <0.001 |

$^{a,b}$indicated the difference within a row was significant (p<0.05)

by essential oil mixes (p<0.05). Bozkurt et al. (2012b) declared that EOM added to layer hen diet significantly enhanced egg production rate and egg weight. Other studies showed no significant change in egg production and egg weight when hens were fed a diet supplemented with EOM (Bozkurt et al., 2012b; Florou-Paneri et al., 2005; Özek et al., 2011). Similarly, Bozkurt et al. (2009) discovered that the addition of EOM at 24 to 48 mg/kg to diet had no effect on the production and weight of eggs for broiler breeders in chickens between the ages of 26 and 46 weeks. This variability in results may be related to the dose of EOs, different application methods, components of EOs or their utilization in different types of poultry.

Eggshell weight increased at week 25 week (p<0.05). At the 30$^{th}$ and 35$^{th}$ weeks of the trial, eggshell weight gradually increased with EOM supplementation, but the difference was not significant (p>0.05). Supplements in drinking water had no effect on albumen and yolk weight (p>0.05) in early period hens at 25 weeks. Eggshell thickness was significantly improved at all periods measured (p<0.05). Adding EOM (0.1, 0.2 or 0.3 mL/L) to drinking water influenced HU at the age of 25 and 30 weeks (p<0.05) The highest HU was obtained in the eggs of layer hens fed drinking water containing 0.3 mL/L EOM. In some studies, it has been observed that the use of different forms of EOs or EOM leads to significant improvements in egg shell weight (Bozkurt et al., 2012b and 2012c). In contrast, Akbari et al. (2015) reported that feeding peppermint and thyme EO had no effect on shell weight in layer hens. In our study, the supplementation of EO significantly increased the eggshell thickness in all weeks (p<0.05). Sim-

**Table 5.** The effects on the internal and external egg quality (mean± standard error)[1] of adding EOMs to layer hens' drinking water

| | Essential oil mixed supplementation (mL/L) | | | | |
|---|---|---|---|---|---|
| **Item** | **0** | **0.1** | **0.2** | **0.3** | **p** |
| **Unit of egg weight** | | | | | |
| 25 wk | 57.18±1.17[b] | 58.13±0.91[ab] | 58.32±0.99[ab] | 60.32±0.83[a] | 0.15 |
| 30 wk | 57.46±0.74 | 57.59±0.68 | 57.85±0.75 | 58.08±1.03 | 0.94 |
| 35 wk | 59.66±0.95 | 59.39±0.90 | 61.64±0.65 | 61.02±0.79 | 0.10 |
| **Eggshell weight (g)** | | | | | |
| 25 wk | 5.95±0.12[b] | 5.97±0.12[b] | 6.03±0.096[b] | 6.37±0.13[a] | 0.04 |
| 30 wk | 6.03±0.12 | 6.04±0.12 | 6.35±0.09 | 6.21±0.11 | 0.12 |
| 35 wk | 5.89±0.09 | 6.09±0.08 | 6.10±0.12 | 5.95±012 | 0.36 |
| **Albumen weight (g)** | | | | | |
| 25 wk | 37.09±0.97 | 38.48±0.79 | 37.97±0.69 | 39.49±0.62 | 0.19 |
| 30 wk | 37.91±0.77 | 36.89±0.84 | 36.08±0.84 | 36.14±0.97 | 0.82 |
| 35 wk | 38.68±0.93[a] | 38.43±0.89[a] | 40.36±0.78[a] | 40.36±0.75[a] | 0.05 |
| **Yolk weight (g)** | | | | | |
| 25 wk | 14.14±0.29 | 13.68±0.17 | 14.31±0.31 | 14.46±0.26 | 0.19 |
| 30 wk | 14.48±0.27[b] | 14.66±0.25[b] | 15.42±0.22[a] | 15.72±0.30[a] | 0.003 |
| 35 wk | 15.09±0.22[a] | 14.86±0.23[b] | 15.18±0.19[a] | 14.71±0.19[b] | 0.05 |
| **Yolk height (mm)** | | | | | |
| 25 wk | 28.94±0.70[a] | 28.96±1.16[a] | 26.12±0.35[b] | 26.20±0.41[b] | 0.004 |
| 30 wk | 26.96±0.27 | 26.65±0.26 | 27.08±0.24 | 26.74±0.29 | 0.65 |
| 35 wk | 27.87±0.33[b] | 29.03±0.27[a] | 29.11±0.28[a] | 28.57±0.25[ab] | 0.01 |
| **Eggshell thickness (mm)** | | | | | |
| 25 wk | 0.32±0.04[a] | 0.28±0.05[b] | 0.29±0.04[b] | 0.31±0.05[a] | 0.035 |
| 30 wk | 0.33±0.05[a] | 0.33±0.04[a] | 0.31±0.05[b] | 0.32±0.05[ab] | 0.001 |
| 35 wk | 0.31±0.03[b] | 0.33±0.03[a] | 0.33±0.08[a] | 0.34±0.06[a] | 0.008 |
| **Haugh unit** | | | | | |
| 25 wk | 78.35±1.22[ab] | 75.99±0.89[b] | 78.72±1.04[ab] | 80.95±1.25[a] | 0.024 |
| 30 wk | 79.26±0.28[b] | 79.15±0.37[b] | 79.86±0.31[ab] | 80.62±0.52[a] | 0.03 |
| 35 wk | 79.69±0.33 | 80.31±0.32 | 80.51±0.58 | 80.80±0.64 | 0.43 |

[a, b]indicated the difference within a row was significant ($p<0.05$)
[1]Means of 20 eggs per treatment

ilarly, Bozkurt et al. (2012b) reported that supplementation of EOM increased egg shell weight, egg shell thickness and shell breaking strength.

Adding oregano EO (50 or 100 mg $kg^{-1}$) to the diet had no effect on HU at the age of 32 weeks (Florou-Paneri et al., 2005). Similarly, Çabuk et al. (2006) observed that adding EOMs or antibiotics to the diet did not significantly affect the egg quality characteristics HU, shell weight or yolk weight in laying quails. Ding et al. (2017) reported that Enviva EO supplementation in layer hens diet had no significant effect on albumen height and HU. However, Xianjing et al. (2017) observed that the use of oregano EO supplementation had no effect on eggshell ratio but found that it did significantly affect yolk ratio, egg shape index and HU.

**Fatty acid compositions**
The mean value of the yolk fatty acids percentage in different treatment groups is shown in Table 6. When compared to the control diet, the proportion of DHA (C22:6 n-3) in the egg yolk was significantly decreased in the treatment groups ($p<0.05$). Palmitoleik acid (16:1) increased in drinking water containing EOM when compared to the control group ($p<0.05$). There was no significant effect on total saturated fatty acids and omega 3 and 6 fatty acid in the experimental groups. To our knowledge, there have been few reports about the effects of EO on the fatty acid composition of egg yolk. In the present study, there were significant differences in the treatment groups in α linolenic acid, linolenic acid, palmitoleic acid, arachidonic acid, lignoceric acid and DHA ($p<0.05$). However, Ding et al. (2017) found that EO in

**Table 6.** The effects on egg yolk fatty acid composition (mean± standard error) of adding EOMs to layer hens' drinking water

| | Essential oil mixed supplementation (mL/L) | | | | |
|---|---|---|---|---|---|
| **Fatty acid (%)** | **0** | **0.1** | **0.2** | **0.3** | **p** |
| C14:0 | 0.28±0.016[b] | 0.29±0.015[ab] | 0.34±0.015[a] | 0.34±0.024[a] | 0.022 |
| C14:1 | 0.005±0.003[b] | 0.008±0.004[b] | 0.016±0.005[b] | 0.033±0.009[a] | 0.004 |
| C15:0 | 0.009±0.005[b] | 0.020±0.006[b] | 0.02±0.006[b] | 0.036±0.002[a] | 0.009 |
| C16:0 | 24.40±0.20[b] | 23.52±0.42[b] | 24.72±0.21[a] | 24.90±0.34[a] | 0.014 |
| C16:1 | 2.67±0.09[c] | 2.86±0.16[b] | 3.23±0.14[ab] | 3.48±0.21[a] | 0.033 |
| C17:0 | 0.21±0.011[b] | 0.21±0.013[b] | 0.21±0.012[b] | 0.28±0.019[a] | 0.005 |
| C17:1 | 0.21±0.05 | 0.18±0.03 | 0.29±0.04 | 0.28±0.053 | 0.272 |
| C18:0 | 9.57±0.20[a] | 9.42±0.35[a] | 8.50±0.38[b] | 7.82±0.30[b] | 0.001 |
| C18:1 | 41.15±0.71 | 41.35±0.49 | 41.32±0.38 | 41.16±0.70 | 0.991 |
| C18:2 n6 | 16.96±0.78 | 17.64±0.41 | 17.42±0.44 | 17.93±0.62 | 0.696 |
| C18:3 n6 | 0.50±0.035 | 0.49±0.052 | 0.48±0.030 | 0.48±0.031 | 0.973 |
| C18:3 n3 | 0.74±0.058[b] | 0.94±0.059[a] | 0.90±0.041[a] | 0.96±0.044[a] | 0.021 |
| C20:0 | 0.17±0.029 | 0.16±0.043 | 0.13±0.025 | 0.10 ± 0.017 | 0.393 |
| C20:1 | 0.25±0.029 | 0.30±0.034 | 0.24±0.018 | 0.27±0.017 | 0.420 |
| C20:2 n6 | 0.22±0.029 | 0.26±0.040 | 0.18±0.022 | 0.19±0.015 | 0.209 |
| C20:3 n3 | 0.20±0.033 | 0.18±0.029 | 0.19±0.018 | 0.17±0.018 | 0.869 |
| C20:4 n6 | 1.68±0.058[a] | 1.45±0.11[ab] | 1.23±0.14[bc] | 1.07±0.13[c] | 0.004 |
| C22:6 n3 | 0.61±0.03[a] | 0.51±0.05[ab] | 0.44±0.06[b] | 0.38±0.05[b] | 0.014 |
| C24:0 | 0.16±0.018[ab] | 0.21±0.03[a] | 0.11±0.014[b] | 0.12±0.014[b] | 0.015 |
| ∑SFA | 34.81±0.33 | 33.84±0.32 | 34.06±0.43 | 33.60±0.51 | 0.187 |
| ∑MUFA | 44.27±0.75 | 44.69±0.48 | 45.10±0.46 | 45.22±0.78 | 0.710 |
| ∑PUFA | 20.91±0.86 | 21.47±0.51 | 20.84±0.38 | 21.18±0.73 | 0.897 |
| ∑UFA | 65.19±0.33 | 66.16±0.32 | 65.94±0.43 | 66.40±0.51 | 0.187 |
| ∑PUFA/∑SFA | 0.60±0.028 | 0.64±0.018 | 0.61±0.016 | 0.63±0.026 | 0.695 |
| ∑UFA/∑SFA | 1.88±0.028 | 1.96±0.027 | 1.98±0.048 | 1.94±0.018 | 0.198 |
| n6 | 19.37±0.81 | 19.84±0.46 | 19.31±0.36 | 19.68±0.67 | 0.911 |
| n3 | 1.54±0.062 | 1.63±0.083 | 1.53±0.049 | 1.50±0.088 | 0.608 |
| n6/n3 | 12.60±0.34 | 12.36±0.49 | 12.72±0.48 | 13.29±0.51 | 0.549 |
| NUTRVALUE | 2.08±0.036[b] | 2.17±0.055[a] | 2.02±0.028[b] | 1.97±0.043[b] | 0.014 |
| ATHERNGINX | 0.17±0.003[a] | 0.16±0.005[a] | 0.15±0.006[ab] | 0.14±0.006[b] | 0.008 |
| THRINDEX | 0.60±0.008 | 0.58±0.007 | 0.58±0.010 | 0.57±0.012 | 0.191 |

n=10
[a,b] indicated the difference within a row was significant ($p<0.05$)
∑SFA= total saturated fatty acids; ∑MUFA= total monounsaturated fatty acids; ∑PUFA= total polyunsaturated fatty acids; n-6: omega-6 fatty acids; n-3: omega-3 fatty acids

layer hen diet had no effect on the fatty acid composition of egg yolk of layer hens. The addition of EOM to the drinking water of layer hens had no effect on the n-6/n-3 ratio in the egg ($p>0.05$). Bölükbaşı et al. (2010) reported that bergamot oil supplementation in layer diets significantly increased the proportion of DHA and n-3 in the egg yolk. While the addition of essential oil to drinking water reduced the SFA concentration, it increased the MUFA concentration, numerically. In agreement with the present study, Ding et al. (2017) reported that SFA concentrations were decreased but PUFA and MUFA concentrations were increased with dietary EO. Bölükbaşı et al. (2008) showed that SFA and PUFA concentrations in leg and breast tissues in broilers were decreased, whereas, MUFA concentrations were increased. To our knowledge, the studies on the addition of essential oils to drinking water are limited. Therefore, further work is needed in order to give more detailed information on this topic in layer hens.

## Conclusion

As a result of supplementing peppermint, oregano, and anise essential oils in the amounts of 0.2 mL/L and 0.3 mL/L to drinking water, there were beneficial effects on performance and egg quality without adverse effects on other parameters.

---

**Ethics Committee Approval:** All experimental protocols adhered to and approved by the guidelines of Animal Ethics Committee of Kafkas University (KAU-HADYEK/2016-032) (Kars, Turkey).

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - Ö.K.; Design - T.Ş., Ö.K.; Supervision - Ö.K.; Resources - M.Ö.; Materials - M.Ö.; Data Collection and/or Processing - Ö.K., B.Ö.; Analysis and/or Interpretation - B.Ö.; Literature Search - Ö.D.A.; Writing Manuscript - Ö.K.; Critical Review - M.Ö.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

**Financial Disclosure:** The present study was supported by Scientific Research Center of Kafkas University (Protocol Number: 2013-VF-68).

## References

**Abdo, Z.M.A., Hassan, R.A., El-Salam, A.A., Helmy, S.A., 2010**. Effect of adding green tea and its aqueous extract as natural antioxidants to laying hen diet on productive, reproductive performance and egg quality during storage and its content of cholesterol. Egyptian Poultry Science Journal 30, 1121-1149.

**Acamovic, T., Brooker, J.D., 2005**. Biochemistry of plant metabolites and their effects in animals. The Proceedings of the Nutrition Society 64, 403-412. [CrossRef]

**Akbari, M., Torki, M., Kaviani, K., 2015**. Single and combined effects of peppermint and thyme essential oils on productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition (6.8±3°C). International Journal of Biometeorology 60, 447-454. [CrossRef]

**AOAC, 1995**. International. Official methods of analysis. Association of Analytical Communities. 2 vols. 16th edition. Arlington, VA, USA: AOAC International.

**Aydın, A., Karaman, M., Cicek, T., Yardibi, H., 2008**. Black cumin (Nigella sativa L.) supplementation into the diet of the laying hen positively influences egg yield parameters, shell quality and decreases egg cholesterol. Poultry Science 87, 2590-2595. [CrossRef]

**Bozkurt, M., Alçiçek, A., Küçükyılmaz, K., Çatlı, A.U., 2009.** Effect of an herbal essential oil mixture on growth, laying traits, and egg hatching characteristics of broiler breeders. Poultry Science 88, 2368-2374. [CrossRef]

**Bozkurt, M., Hippenstiel, F., Abdel-Wareth, A.A.A., Kehraus, S., Küçükyılmaz, K., H-Südekum, K., 2014.** Effects of selected herbs and essential oils on performance, egg quality and some metabolic activites in laying hens- a review. European Poultry Science 78, 1-15.

**Bozkurt, M., Küçükyilmaz, K., Çatli, A.U., Çınar, M., Bintaş, E., Çöven, F., 2012a**. Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. Poultry Science 291, 1379- 1386. v

**Bozkurt, M., Küçükyilmaz, K., Pamukçu, M., Çabuk, M., Alçiçek, A., Çatli, A.U., 2012b**. Long-term effects of dietary supplementation with an essential oil mixture on growth and laying performance of two layer strains. Italian Journal of Animal Science 11**,** 23-28. [CrossRef]

**Bozkurt, M., Tokuşoglu, Ö., Küçükyilmaz, K., Akşit, H., Çabuk, M., Çatli, A.U., Seyrek, K., Çinar, M., 2012c.** Effects of dietary mannan-oligosaccharide and herbal essential oil blend supplementation on performance and oxidate stability of eggs and liver in laying hens. Italian Journal of Animal Science 11, 223-229. [CrossRef]

**Bölükbaşı, Ş.C., Erhan, M.K., Kaynar, O., 2008**. The effect of feeding thyme, sage and rosemary on laying hen performance, cholesterol and some proteins ratio of egg yolk and *Escherichia coli* count in feces. Archiv für Geflügelkunde 73, 167-172.

**Bölükbaşı, Ş.C., Erhan, M.K., Ürüşan, H., 2010.** The effects of supplementation of bergamot oil (Citrus bergamia) on egg production, egg quality, fatty acid composition of egg yolk in laying hens. The Journal of Poultry Science 47, 163-169. [CrossRef]

**Carpenter, K.J., Clegg, K.M., 1956.** The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. Journal of The Science of Food and Agriculture 7(1), 45-51. [CrossRef]

**Çabuk, M., Bozkurt, M., Alçiçek, A., Çatlı, A.U., Başer, K.H.C., 2006.** Effect of a dietary essential oil mixture on performance of laying hens in the summer season. South African Society for Animal Science 36, 215-221.

**Çabuk, M., Eratak, S., Alçiçek, A., Bozkurt, M., 2014.** Effects of herbal essential oil mixture as a dietary supplement on egg production in quail. The Scientific Word Journal 1, 573470. [CrossRef]

**Dawson, B. and Trap, R.G. 2001.** Basic and Clinical Biostatistics, 3rd ed. Lange Medical Books/McGraw-Hill Medical Publishing Division New York.

**Ding, X., Yu, Y., Su, Z., Zhang, K., 2017**. Effects of essential oils on performance, egg quality, nutrient digestibility and yolk fatty acid profile in laying hens. Animal Nutrition 3, 127-131. [CrossRef]

**Ertaş, O.N., Güler, T., Çiftçi, M., Dalkılıç, B., Simsek, U.G., 2005.** The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. International Journal of Poultry Science 4(11), 879-884. [CrossRef]

**Espina, L., Pagan, R., Lòpez, D., Garzìa-Gonzalo, D., 2015.** Individual constituents from essential oils inhibit biofilm mass production by multi-drug resistant *Staphylococcus aureus*. Molecules 20, 11357-11372. [CrossRef]

**Florou-Paneri, P., Nikolakakis, I., Giannenas, I., Koidis, A., Botsoglou, E., Dotas, V., Mitsopoulos, I., 2005.** Hen performance and egg quality as affected by dietary oregano essential oil and α-tocopheryl acetate supplementation. International Journal of Poultry Science 4, 449-454. [CrossRef]

**Hippenstiel, F., Abdel-Wareth, A.A.A., Kehraus, S., Südekum, K.H., 2011.** Effect of selected herbs and essential oils and their active components on feed intake and performance of broilers-a review Archiv für Geflügelkunde 75, 226-234.

**Khan, S.H., Sardar, R., Anjum, M.A., 2007.** Effects of dietary garlic on performance snd serum and egg yolk cholesterol concentration in laying hens. Asian Journal of Poultry Science 1, 22-27. [CrossRef]

**Mooyottu, S., Kollanoor-Johny, A., Flock, G., Bouillaut, L., Upadhyay, A., Sonenshein, A.L., Venkitanarayanan, K., 2014.** Carvacrol and trans-cinnamaldehyde reduce clostridium difficile toxin production and cytotoxicity in vitro. International Journal of Molecular Science 15, 4415-4430. [CrossRef]

**NRC, 1994**. Nutrient Requirements of Poultry. Nineth Revised Edition, National Academy Press, Washington, DC.

**Olgun, O., Yıldız, A.Ö., 2014.** Effect of dietary supplementation of essential oils mixture on performance, eggshell quality, hatchability, and mineral excretion in quail breeders. Environmental Science and Pollution Research 21, 13434-13439. [CrossRef]

**Özek, K., Wellnann, K.T., Ertekin, B., Tarım, B., 2011.** Effects of dietary herbal essential oil mixture and organic acid preparation on laying traits, gastrointestinal tract characteristics, blood parameters and immune response of laying hens in a hot summer season. Journal of Animal and Feed Science 20, 575-586. [CrossRef]

**Radwan, N.L., Hassan, R.A., 2008.** Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. International Journal of Poultry Science 7, 134-150. [CrossRef]

**Simopoulos, A.P., Salem, N., 1992.** Egg yolk as source of long-chain polyunsaturated fatty acids in infant feeding. The American Journal of Clinical Nutrition 55, 411-414. [CrossRef]

**Williams, P., Losa, R., 2001.** The use of essential oils and their compounds in poultry nutrition. World's Poultry Science Journal 17, 14-15.

**Xianjing, H., Hao, D., Liu, C., Zhang, X., Xu, D., Xu, X., Wang, J., Wu, R., 2017.** Effect of supplemental oregano essential oils in diets on production performance and relatively intestinal parameters of laying hens. American Journal of Molecular Biology 7, 73-85. [CrossRef]

**Yi, H., Hwang, K.T., Regenstein, J.M., Shin, S.W., 2014.** Fatty acid composition and sensory characteristics of eggs obtained from hens fed flaxseed oil dried Whitebait and/ or fructo-oligosaccharide. Asian-Australasian Journal of Animal Science 27, 1026-1034. [CrossRef]