



### Research Article

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Ibrahim Halil GÜMÜS<sup>1,2</sup> , Ezgi SENSES<sup>2</sup> , Arda SÖZCÜ<sup>1\*</sup> 

<sup>1</sup>Tekinler Agriculture Company, Izmir, 35170, Türkiye

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Uludağ University, Bursa, 16100, Türkiye

## Improving Egg Production, Quality and Eggshell Hygiene by Supplementation of Sage (*Salvia officinalis*) and Lavender (*Lavandula angustifolia*) Essential Oils into Drinking Water #

### ABSTRACT

**Objective:** This study was performed to evaluate the effects of sage (*Salvia officinalis*) and lavender (*Lavandula angustifolia*) essential oils (EO) supplementation into drinking water on egg production, quality and eggshell hygiene in laying hens.

**Material and Methods:** A total of 300 Nick Brown laying hens between 30 and 38 of age were used in a completely randomized design in three treatments and ten replicates. The treatments consisted of: control group, sage EO, lavender EO supplementation group. EOs were supplemented into drinking water (0.3 mL/L).

**Results:** A lower value of egg production, egg weight, egg mass and deteriorations in feed conversion ratio, eggshell breaking strength and thickness were observed in lavender EO group (P<0.05). However, the number of total aerobic mesophilic bacteria, *Coliform spp.*, and yeast and mold of eggshell was reduced by supplementation of sage and lavender EO, compared to the control group (P<0.001).

**Conclusion:** Current findings showed that the supplementation of sage EO could be effective for improving egg production, and eggshell strength; however lavender EO negatively affected the productivity. A specific beneficial effect of both sage and lavender EOs observed for eggshell hygiene, when compared to the control group.

**Keywords:** Egg production, feed conversion ratio, eggshell breaking strength, eggshell hygiene, *Salvia officinalis*, *Lavandula angustifolia*, essential oil

## İçme Suyuna Adaçayı (*Salvia officinalis*) ve Lavanta (*Lavandula angustifolia*) Esansiyel Yağ İlavesiyle Yumurta Verimi, Kalitesi ve Yumurta Kabuk Hijyeninin Artırılması

### ÖZ

**Amaç:** Bu çalışma, yumurtacı tavuklarda içme suyuna adaçayı (*Salvia officinalis*) ve lavanta (*Lavandula angustifolia*) esansiyel yağları ilavesinin yumurta verimi, kalitesi ve yumurta kabuk hijyeni üzerine etkilerinin değerlendirilmesi için yürütülmüştür.

**Materyal ve Metot:** Tesadüf parselleri deneme deseninde yürütülen bu çalışma 300 adet Nick Brown yumurtacı tavuk ile 30-38 haftalık yaş döneminde, üç deneme grubu ve on tekrerrür (her tekrerrürde on adet tavuk) ile tamamlanmıştır. Deneme grupları: kontrol grubu, adaçayı esansiyel yağı ilavesi, lavanta esansiyel yağı ilavesi. Esansiyel yağlar içme suyuna 0.3 mL/L düzeyinde ilave edilmiştir.

**Bulgular:** Lavanta esansiyel yağı ilave edilen deneme grubunda yumurta verimi, yumurta ağırlığı ve yumurta kütlelerinin daha düşük olduğu, yemden yararlanma oranı, kabuk kırılma direnci ve kabuk kalınlığında kötüleşme meydana geldiği gözlemlenmiştir (P<0.05). Ancak, kontrol grubu ile kıyaslandığında, adaçayı ve lavanta esansiyel yağları ilavesi kabuk yüzeyindeki toplam aerobik mezofilik bakteri, *Coliform spp.* ve küf-maya popülasyonunu azaltıcı yönde etki etmiştir (P<0.001).

**Sonuç:** Elde edilen bulgular adaçayı esansiyel yağı ilavesinin yumurta verimi ve kabuk kırılma direncini artırma için etkili olabileceğini, ancak lavanta esansiyel yağı ilavesinin ise performansı olumsuz yönde etkilemiştir. Kontrol grubu ile kıyaslama yapıldığında, hem adaçayı hem de lavanta esansiyel yağının kabuk hijyeni üzerine özel bir olumlu etkisi olduğu gözlemlenmiştir.

**Anahtar Kelime:** Yumurta verimi, yemden yararlanma, kabuk kırılma direnci, kabuk hijyeni, *Salvia officinalis*, *Lavandula angustifolia*, esansiyel yağ

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## INTRODUCTION

In animal production, the usage of antibiotics as growth promoter parameters has been banned in many countries, mainly in European Union countries, since 2006 (Butaye et al., 2000; O'Neill, 2016). Therefore, a huge interest has been focused on feed additives which could have potential to be new alternative instead of antibiotics for beneficial effects in poultry production (Garcia et al., 2019; Gholami-Ahangaran et al., 2022; Ürüşan, 2023). Among these alternatives, herbal additives, also called as phytogetic feed additives, have been increasingly gained importance due to their possible stimulating effects for productive performance, physiological and health status of birds (Gholami-Ahangaran et al., 2022).

Aromatic and medicinal plants, spices could be used as phytogetic additives in different forms of dried plant, plant extracts, essential oils (EO) as singly or combined forms, and supplemented into feed or drinking water (Bozkurt et al., 2014). EOs are produced by plants and have aromatic properties with fragile and volatile compounds. The chemical composition, and the content and level of active compounds could vary depending on plant species (Hippenstiel et al., 2011). It is known that these herbal sources are a rich source of biologically active substances, containing more than 8000 phytochemicals, such as phenols, flavonoids, saponins, tannins, essential oils (Yadav et al., 2016). Depending on these phytochemicals, some beneficial effects, for example anti-oxidative, anti-fungal, anti-carcinogenic, anti-inflammatory, and antimicrobial effects, could be provided (Bahmani et al., 2014; Gholami-Ahangaran et al., 2020).

Sage (*Salvia officinalis*) is a member of the *Labiatae/Lamiaceae* plant family, and easily grow in many areas having ideal conditions (Bahtiyarca Bagdat et al., 2017). It is largely used in human nutrition due to its therapeutic ability (Alekish et al., 2017), comprising phytochemical compounds, alkaloids, carbohydrates, fatty acids, asparagine, glutamine, carotene, steroids, poly acetylenes, terpenes/terpenoids, phenolic substances (e.g., tannins, coumarins, flavonoids), glycosidic derivatives (saponins, cardiac glycosides, flavonoid glycosides,), and waxes (Ghorbani and Esmailizadeh, 2017; Hrebień-Filisińska and Bartkowiak, 2021). The EO of sage mainly includes the monoterpenes eucalyptol, alpha- and beta thujone, borneol (Landjev, 2010). These compounds could provide pharmacological effects, as anti-inflammatory, anti-cancer and antioxidant, as well as a neuroprotective and wound-healing effects (Fu et al., 2013). According to the potential beneficial effects of sage, previous studies investigated the potential effects of sage leaves and/or extracts in poultry nutrition. Some significant improvements were observed in sperm quality and productivity in roosters (Ommati et al., 2013), average daily weight gain (Levkut et al., 2010), and blood biochemical parameters (Piesova et al., 2012) as well as for reducing the total number of Enterobacteriaceae (Rasouli et al., 2020; Galamatis et al., 2021).

Lavender (*Lavandula angustifolia*), is also known as Common Lavender, French lavender, English Lavender, Garden Lavender, is one of the important aromatic and medicinal plants which is a flowering plant from the *Lamiaceae* family. The roots, leaves and fruits of the lavender could be used as medicinal plant, as well as essential oil could be produced by fresh flowers and/or the inflorescence stage. It has been highlighted that lavender EO contains more than 100 components (Martucci et al., 2015); the main compound of lavender EO is lavandula (1%–3%) which is rich in linalyl acetate and linalool. The lavender EO also includes rosmarinic acid and coumarin (Lis-Balchin, 2002; Marín et al., 2016). On the other hand, Bakhsha et al. (2014) reported the components of lavender EO are mainly; carvacrol (26.2%), limonene (19.6%), 1,8-cineole (11.8%), and the minor componenets are terpinen-4-ol (7.6%), spathulenol (4.9%),  $\alpha$ -pinene (4.2%), p-cymene (4.2%), caryophyllene oxide (2.7%) and terpinolene (2.6%). Lavender is largely used in food, drugs, cosmetics sectors (Danh et al., 2013). It is well known that the lavender has strong antimicrobial properties as nematicidal, antifungal, anti-mite, and antiviral activity (Adaszyńska-Skwirzyńska et al., 2021). Depending on these effects, lavender could potentially inhibit the proliferation of pathogenic bacteria's, and stimulates the growth of the beneficial microbiota (Alali et al., 2013; De Rapper et al., 2016). Besides it has therapeutic effects such as anti-oxidative, anti-depressant, anti-inflammatory, analgesic, tranquilizing, hypnotic, and anti-carcinogenic activity and also antioxidant and immune-stimulating effects (Carrasco et al., 2016; De Rapper et al., 2016; Adaszyńska-Skwirzyńska and Szczerbińska, 2019).

According to available knowledge, the hypothesis of this study is the supplementation of sage (*Salvia officinalis*) and lavender (*Lavandula angustifolia*) EO into drinking water would be effective for enhancement of productive performance, egg quality parameters, and providing an inhibition of eggshell microbiota.



## MATERIAL and METHODS

A total of 300 Nick Brown genotype laying hens at 30 weeks of age were used in the experiment. This study was carried out in research house of a commercial facility located in İzmir, Turkey. Ten sub-groups were created for each experimental group and each sub-group contained 10 laying chickens

The laying hens were divided into control group and two experimental groups with EO supplementation including sage (*Salvia officinalis*) and lavender (*Lavandula angustifolia*) supplementation. The drinking water was supplemented with sage and lavender EOs (Greenext Global Organic Chemistry, İzmir, Turkey) at a concentration of 0.3 mL/L between 30 and 38 weeks of age with daily basis. Each experimental group was equipped with 10 liter tanks that were connected with nipple drinkers. The active components of sage and lavender EOs, according to the information given by the manufacture, are shown in the Table 1. A standard commercial layer diet (17.8% CP and 2815 ME kcal/kg) was used. The basal ration was formulated mainly with soybean meal and maize according to the National Research Council (NRC, 1994).

Table 1. The active compounds of sage (*Salvia officinalis*) and lavender (*Lavandula angustifolia*) EOs

Çizelge 1. Adaçayı (*Salvia officinalis*) ve lavanta (*Lavandula angustifolia*) esansiyel yağlarının aktif bileşenleri

EOs	Compounds	%
Lavender EO	L-Linalool	37.42
	Linalyl acetate	26.38
	Terpinene	4.59
	Caryophyllene	3.37
	Lavandulyl acetate	2.90
Sage EO	1,8-Cineole	37.56
	Camphor	24.26
	β-Caryophyllene	8.15
	Borneol	8.14
	Thymol	2.85

The nutrient content and content of the diet was expressed in Table 2. Feed and water were offered ad-libitum during experimental period. All hens were provided with a constant lighting schedule applied as 16 hours lighting period and 8 hours' dark period.

Table 2. Composition and nutrient content of the basal diet

Çizelge 2. Rasyon bileşimi ve besin madde içeriği

Ingredients	%	Nutrient content	
Corn	62.5	Metabolizable energy (kcal/kg <sup>-1</sup> )	2823
Soybean meal, 48%	22.8	Crude protein (%)	17.8
Soybean oil	2.0	Calcium (%)	4.15
Limestone	10.0	Available phosphorus (%)	0.55
NaCl	0.35		
Di-calcium phosphate	1.6		
L-Lysine-HCl	0.05		
DL-methionine	0.20		
Premix*	0.50		

\* Vitamin premix provided per kg of diet: Vitamin A: 2.40 mg; Vitamin D3: 75.00 µg; Vitamin E: 5.00 mg; Vitamin K3: 2.20 mg; Vitamin B1: 1.50 mg; Vitamin B2: 4.00 mg; Vitamin B3: 8.00 mg; Vitamin B5: 35.00 mg; Vitamin B6: 2.50 mg; Vitamin B9: 0.50 mg; Vitamin B12: 10.00 µg; Vitamin H2: 0.15 mg; Choline: 468.70 mg; Mn: 80.00 mg; Fe: 75.00 mg; Zn: 64.00 mg; Cu: 6.00 mg; Se: 0.30 mg.

### Laying Performance Parameters

The body weight of hens was individually weighed at 30 and 38 weeks of age. The number of eggs was recorded as daily basis by collecting manually in each pen. The egg production was given as hen-day egg production (HDEP), whereas daily feed intake and egg weight were monitored on weekly basis. Egg mass was given as ratio between the egg weight with HDEP. Feed conversion ratio were calculated by rating between feed intake and egg mass.



### Egg Quality Parameters

At 38 weeks of age, to determine the egg quality, 30 eggs per experimental group (randomly selected 3 eggs from each replicate) were subjected to exterior and interior quality analysis. The eggs were weighed with  $\pm 0.01$  g precision, and then measured for the length and width by digital caliper ( $\pm 0.01$  mm precision, Mitutoyo, 300 mm, Neuss, Germany). These values were used for calculation of egg shape index according to the formula given by Reddy et al. (1979):

$$\text{Egg shape index (\%)} = (\text{Egg width}/\text{Egg length}) \times 100$$

Eggshell breaking strength was measured by using an eggshell force reader machine (Egg Force Reader, Orka Food Technology, Israel). Then, the eggs were broken on a glass table to measure interior egg quality parameters. Egg yolk diameter, albumen width and length, were measured with digital caliper (Mitutoyo, 300 mm, Neuss, Germany), whereas the height of yolk and albumen were determined by using a tripod micrometer. The formulas given at below were used to calculate yolk index, albumen index, and Haugh unit (Heiman and Carver, 1936; Haugh, 1937; Funk, 1948):

$$\text{Yolk index} = (\text{Yolk height}/\text{Yolk diameter}) \times 100$$

$$\text{Albumen index} = (\text{Albumen height}/(\text{Albumen length} + \text{Albumen width})/2) \times 100$$

$$\text{Haugh unit} = 100 \times \log(\text{Albumen height} + 7.57 - 1.7 \times \text{Egg weight}^{0.37})$$

Then, egg yolk color was measured with DSM egg yolk color fan (Roche Ltd., Switzerland), ranging from the lightest (score 1) to the darkest yolk color (score 15). The yolk weight was determined by weighing with  $\pm 0.01$  g precision. The eggshells were washed and exposed a drying process for 24 h at 105°C in an oven (Nuve FN-500, Ankara, Turkey), and then weighed. Albumen weight was determined by subtracting the sum of yolk and shell weights from the egg weight. The thickness of eggshell was determined of measuring the thickness of the upper, middle, and lower end of the shell egg using a special caliper, and it was given as the average thickness of these three parts of the shell.

### Microbial analysis

The eggshell samples (n=5 samples from each experimental group) with an amount of 10 g were collected and then out in a sterile containers containing 50 mL of phosphate buffered saline solution, and homogenized for 2 minutes with a vortex. To numerate of bacteria in eggshell samples, the decimal dilutions were made in sterile test tubes with containing 9 ml of phosphate buffered saline (0.1%).

For enumeration of total mesophilic aerobic bacteria and coliforms, Plate Count Agar and Violet Red Bile Lactose Agar (Merck, Germany) were used. From each dilution, duplicate pour plates were created. For 24 to 48 hours, plates were incubated at 37 °C. Coliform bacteria were counted as pink-red colonies from the appropriate dilution, and all colonies from the appropriate dilution were counted as mesophilic aerobic bacteria (Harrigan, 1998).

Potato Dextrose Agar with 10% tartaric acid added was used for mold and yeast counts, and duplicate pour plates were created from each dilution. After five days of incubation at 22 °C, colonies with a soft mucoid consistency with oval or rounded edges were classified as yeast, while colonies with a mycelium that resembled "puffy cotton" were classified as mold (Andrew, 1992).

### Statistical Analysis

Data obtained in the study was subjected to statistical analysis using the general linear model (GLM) procedure in a completely randomized design in three treatments and ten replicates (ten birds per replicate, Minitab 17).

The following statistical model was used to determine the effects of the treatment:

$$Y_{ij} = \mu + \alpha_{ij} + e_{ij}$$

where  $\mu$  = general mean,  $e_{ij}$  = random error,  $\alpha_i$  = effect of EOs treatments, and  $Y_{ij}$  = response variable.

The means were given with the standard error of the mean (SEM). The differences among treatment groups were analyzed by Duncan's multiple range tests. Differences were considered significant at  $P \leq 0.05$ .



## RESULTS and DISCUSSION

The effects of sage and lavender EO supplementation into drinking water on performance parameters were presented on Table 3. Performance data indicated that there were no significant differences between feed consumption, and body weight of laying hens in the experimental groups ( $P>0.05$ ). During experimental period, HDEP, egg weight, egg mass and feed conversion ratio were found to be improved in the sage EO supplementation and control groups, compared to the lavender EO supplementation group ( $P<0.01$ ).

Table 3. The effects of sage (*Salvia officinalis*) and lavender (*Lavandula angustifolia*) EO supplementation into drinking water on performance parameters

Çizelge 3. İçme suyuna adaçayı (*Salvia officinalis*) ve lavanta (*Lavandula angustifolia*) esansiyel yağı ilavesinin performans parametreleri üzerine etkileri

Performance parameters	Experimental groups			P-value
	Control	Sage EO	Lavender EO	
Hen-day egg production (%)	86.6 ± 0.6 <sup>ab</sup>	87.3 ± 0.6 <sup>a</sup>	85.6 ± 0.6 <sup>b</sup>	0.035
Egg weight (g)	63.9 ± 2.7 <sup>a</sup>	63.6 ± 2.7 <sup>a</sup>	60.30 ± 2.3 <sup>b</sup>	<0.001
Egg mass (g/hen/day)	54.4 ± 1.4 <sup>a</sup>	53.7 ± 1.2 <sup>a</sup>	51.0 ± 1.0 <sup>b</sup>	<0.001
Feed consumption (g/hen/day)	121.7 ± 12.2	117.8 ± 10.2	119.4 ± 14.1	0.217
Feed conversion ratio (feed/egg)	2.24 ± 0.03 <sup>b</sup>	2.19 ± 0.04 <sup>b</sup>	2.34 ± 0.02 <sup>a</sup>	0.002
Body weight at 30 wks of age (g)	1827.5 ± 65.3	1840.3 ± 79.7	1833.0 ± 67.0	0.212
Body weight at 38 wks of age (g)	1771.8 ± 81.4	1817.5 ± 94.6	1833.5 ± 79.6	0.068

a, b—values in rows with different letters differ significantly ( $P < 0.01$ )

The supplementation of sage EO provided an increment in HDEP (87.3%), whereas the lavender EO resulted in a lower of HDEP (85.6%), compared to the control group (86.6%). Current results agree with previous studies performed by Saleh et al. (2021) and Todorava et al. (2024) who found an increment in egg production with sage supplementation into feed. This stimulating effect was attributed to the content of phytoestrogens in sage, which possibly affected the oviduct development and subsequently provided an increment in egg productivity.

In the control and sage EO supplementation group, an increment in egg weight and egg mass was observed and this increment possibly provided an improvement in feed conversion ratio. This hypothesis was earlier confirmed by Nobakht et al. (2011), Sayedpiran et al. (2011). Despite no significant differences were observed for feed consumption between experimental groups, a more efficient of feed conversion ratio was observed in the control (2.24) and sage EO supplementation group (2.19) than the lavender EO supplementation group (2.34;  $P<0.01$ ). When compared to the control group, sage EO provided any beneficial effect for feed conversion ratio, but lavender EO supplementation caused deterioration in feed conversion ratio, and also other performance parameters including HDEP, egg weight and egg mass. In contrast, in a previous study dietary supplementation of lavender EO with an amount of 350 mg/kg feed provided a significant improved in feed conversion ratio in broilers (Nasiri-Moghaddam et al., 2012). On the other hand, the supplementation of Lavender stoechas (200, 400 and 600 ppm) provided any improvement in feed conversion ratio in laying hens in another study (Salari et al., 2014).

When compared to the control group, laying hens in sage and lavender EO supplementation groups showed a tendency to gain more body weight, despite the non-significant differences between for body weight at 38 weeks of age in the experimental groups. On the other hand, previous studies reported an increment in body weight when the diet was supplemented with different essential oils (Hernandez et al., 2004; Traesel et al., 2011). Hernandez et al. (2004) explained this effect by appetite stimulation and improvement in digestive processes by sage supplementation.

These contradictions between the data reported in literature could be originated from the differences origin, content, used amount of the essential oil, supplementation type (into feed or drinking water), genotype, age, and health status of birds. Furthermore, some authors highlighted that the supplementation amount of essential oils could cause some toxic effects, especially with higher amount of essential oils, or amount of active compounds in essential oils (Krishan and Narang, 2014). Torki et al., (2021) claimed that a significant decline in egg production was observed in supplementation of *Lavandula angustifolia* and *Mentha spicata* (250 mg/kg lavender + 250 mg/kg mint) essential oil in laying hens due to possible toxicity of EOs. Therefore, more



toxicological studies focused on essential oils should be carried out to understand the metabolic and physiological pathways of essential oils. Our results clearly suggested that lavender EO possibly caused an inhibiting effect on performance parameters in laying hens.

In this study, the differences observed in the weight of yolk and albumen could be attributed to the egg weight. However, the percentage of yolk was found to be the lowest in lavender EO supplementation group (32.4%), then the control (35.1%) and sage EO supplementation (34.4%) groups ( $P<0.01$ ). These findings showed a consistence with the negative effect of lavender EO on egg production performance, which might be originated from a possible toxic effect of lavender. Eggshell breaking strength and eggshell thickness was found to be the lowest in the lavender EO group (1.871 kg/cm<sup>2</sup> and 0.539 mm respectively,  $P<0.05$ ). As mentioned previously, some of plant additives might be effective for regulation of calcium metabolism via stimulation of hormone secretion, and thereby an improvement in eggshell quality (Esenbuga and Ekinci, 2023). Furthermore, eggs obtained from laying hens in the sage EO group had darker color of yolk compared to the control and the lavender EO groups. This could be an indicator for sage as its effect for increment in transition of the pigments to the yolk, which previously suggested by Esenbuga and Ekinci, (2023).

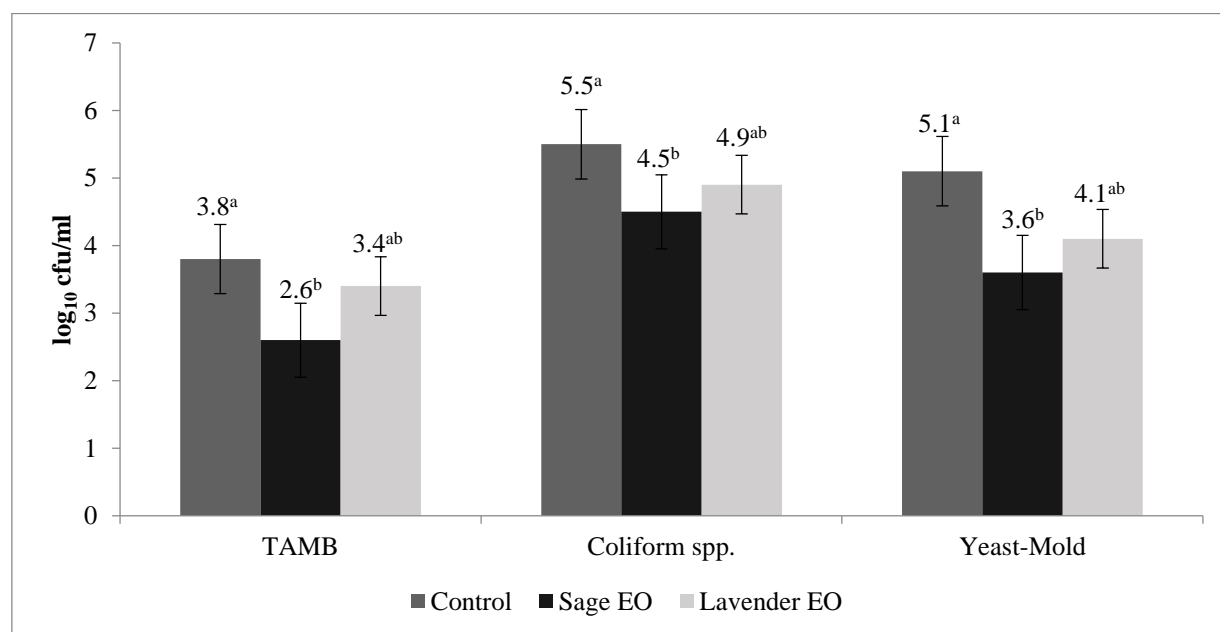


Figure 1. Eggshell bacterial load by sage (*Salvia officinalis*) and lavender (*Lavandula angustifolia*) EO supplementation groups into drinking water, Bars represent mean  $\pm$ SE. ( $P<0.05$ )

Şekil 1. İçme suyuna adaçayı (*Salvia officinalis*) ve lavanta (*Lavandula angustifolia*) esansiyel yağı ilave edilen gruplarda yumurta kabuğu bakteri yükü, Çubuklar ortalama  $\pm$ Standart hata'yı temsil etmektedir. ( $P<0.05$ )

As shown in Figure 1, total number of total mesophilic aerobic bacteria's, *Coliform spp.*, and yeast and mold in eggshells were found to be lower in the sage and lavender EO supplementation groups than the control group ( $P<0.01$ ). This could be attributed to the antibacterial properties of both sage and lavender. A similar effect was also reported by Galamatis et al. (2021) who found a reduction in the total number of Enterobacteriaceae in eggshells by diet supplementation of 1.0% *Salvia officinalis* group compared to the control and supplementation of 0.5% *Salvia officinalis* groups.

## CONCLUSION

Based on the current findings, it could be concluded that the supplementation of sage EO into drinking water (0.3 mL/L) might improve egg production, egg weight, feed conversion ratio and eggshell strength, and indeed it is more effective than lavender EO supplementation. On the other hand, both of sage and lavender EO could be used for providing eggshell hygiene by decreasing the microbial population of eggshell. However, regarding the decline in performance and quality parameters in lavender EO group, sage EO could be potentially recommended as phytobiotics additive in laying hens.



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