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Original Article

Effects of Lavender (Lavandula Angustifolia) Essential Oil on Fattening Performance, Meat Quality, Serum Antioxidant Enzymes, Gut Microbiota and Intestinal Histomorphology in Japanese Quails

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■Keywords

Fatty acid, lavender, meat, microbiota, quail.

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ABSTRACT

This study examined the effects of lavender essential oil on performance, meat quality, microbial load, fatty acid profile and gut microbiota in quails. In the study, 200 quails (*Coturnix coturnix Japonica*) were divided into 4 groups and 5 subgroups. The groups consisted of a control group (0 mg/kg feed) and three lavender essential oil groups, namely Lav125 (125 mg/kg feed), Lav250 (250 mg/kg feed), and Lav500 (500 mg/kg feed). In terms of body weight change, Lav500 group had the best results after the control group ($p>0.05$). It was observed that, compared to the control group, pH levels were high ($p<0.05$) in Lav250 and Lav500 groups on the 9th day of storage. The effect of storage time on malondialdehyde (MDA) was used as a parameter of lipid peroxidation in meat, and the Lav250 and Lav500 groups presented lower concentrations as compared to the control group ($p<0.05$). In this study, the addition of lavender essential oil to the diet enriched the concentrations of n-3 and n-6 polyunsaturated fatty acids (PUFA). Moreover, the height of villi in the duodenum and jejunum, and consequently absorption, increased significantly in the Lav500 group as compared to the control group. The concentration of MDA, a blood serum antioxidant enzyme, decreased with the addition of lavender oil. Additionally, lavender essential oil added to quail diets was observed to increase the number of *Lactobacillus spp.* (probiotic bacteria) colonies ($p<0.05$).

INTRODUCTION

Poultry meat has many desirable nutritional properties, such as low lipid contents and a relatively high concentration of polyunsaturated fatty acids. Increasing the degree of saturation with dietary components increases the sensitivity to oxidation of chicken meat (Enberg *et al.*, 1996; Botsoglou *et al.*, 2002). Due to its polyunsaturated fatty acid contents, poultry meat is relatively susceptible to oxidative degradation (Brenes & Roura, 2010). Lipid oxidation decreases meat shelf life, and affects both product quality and consumer preferences due to the loss of color, smell and taste (Botsoglou *et al.*, 2003). The addition of antibiotics to poultry diets results in residues and development of antimicrobial resistance. Today, aromatic plants and their essential oils, which do not cause residue problems, are alternative plant sources. Plant extracts are natural products that have protective functions against pathogenic microorganisms, acting as phytobiotics (Zeng *et al.*, 2015). Lavender (*L. angustifolia Mill. subsp. Angustifolia)* is a bloom plant of the Lamiaceae family native to Mediterranean countries (Cavanagh & Wilkinson, 2002). Lavender essential oil is obtained from the lavender plant, found in pharmacies and also used in cosmetics (Kirimer *et al.*, 2017). The composition of the oil is depending on the genotype, the conditions of the plant, the mode of production and morphological features, as well as the climate. (Prusinowska & Śmigielski, 2014).

Lavender essential oil is a bioactive substance with a specific odor. It is a multicomponent mixture of terpenoid compounds (monoterpenes, sesquiterpenes and their oxygen derivatives) in terms of its chemistry (Carrasco *et al.*, 2016). Hydrocarbons (e.g., myrcene, α-pinene, and caryophyllene), alcohols (e.g., linalool, α-terpineol, and borneol), ketones (e.g., camphor, carvone, and eukarvone), esters (ex., linalool acetate, lavender-dulyl acetate, and geranyl acetate), aldehydes (e.g. neral), oxides (e.g., caryophyllene oxide) and ethers (e.g., eucalyptol) be a part of this mixture (Lis-Balchin, 2002). In addition to these compounds, there are coumarins and organic acids (Prusinowska & Śmigielski, 2014). It were reported that the use of essential oils in the diet improved the taste and aroma of eating, increased feed consumption, regulated digestive function, changed the microflora or the gastrointestinal system of the animal, and as a result, improved the rate of conversion for growth and feed, which is especially important for decreasing feed costs (Adaszyńska-Skwirzyńska & Szczerbińska, 2019, Barbarestania Yarmohammadi *et al.*, 2020). However, there are studies reporting that the application of essential oils as growth substitutes does not always improve performance parameters, sometimes even worsening them (Kırkpınar *et al.*, 2011; Saleh *et al.*, 2014; Zeng *et al.*, 2015). The reasons for this are probably wrong oil concentrations or short application times. Differences in the reported results may be due to the inclusion of weak chicks or the influence of environmental factors such as hygiene, lighting, or equipment. Additionally, studies on rats and humans reported that it was antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory and antispasmodic properties (Yang *et al.*, 2010; Prusinowska & *Ś*migielski 2014; Carrasco *et al.*, 2016; and Giovannini *et al.*, 2016). It was reported in studies that lavender had immunostimulant, anxiolytic, sedative, hypnotic, analgesic and anticonvulsant effects, and that it can have positive effects on the mental health of humans (Ghelardini *et al.*, 1999; Sasannejad *et al.*, 2012; Prusinowska & Śmigielski, 2014). There are studies investigating the effect of essential oils on the intestinal microflora of poultry (Erhan *et al.*, 2012; Hong *et al.*, 2012; Zeng *et al.*, 2015). *In vivo* studies reported that dietary supplementation with essential oil produces inhibitory effects against pathogens such as *Clostridium spp.*, *Salmonella spp.*, *Escherichia coli,* and *Coccidia spp* (Hong *et al.*, 2012; Zeng *et al.*, 2015).

The literature includes a limited number of studies on the effects of the proper usage and dosage of lavender essential oil on the growth performance, meat and fatty acid profile, and gut microbiota of farm animals. Recent studies include lavender added to the drinking water (Adaszyńska-Skwirzyńska & Szczerbińska, 2019) or to the diet at low concentrations (Küçükyılmaz *et al.,* 2017). The design of this study was to dissolve lavender essential oil in ethyl alcohol and add it to the feed by spraying. The general aim of this study was to investigate the positive effects of lavender essential oil on fattening performance, meat quality, microbial load, and fatty acid profile, as well as on inhibiting pathogenic bacteria and fostering probiotic bacteria in the intestinal flora.

MATERIAL AND METHODS

Animals and maintenance

A total of 200 15-day-old mixed sex Japanese quails (*Coturnix coturnix japonica*) were divided into 4 groups for 35 days. Each group was divided into 5 subgroups. The study was carried out in subgroups with a total of 20 animals (height: width: length (20 cm:45 cm:90 cm). Lavender (*L. angustifolia Mill. subsp. Angustifolia*) oil was obtained from Afyonkarahisar Medicinal and Itri Plants Center. Lavender stock solution was prepared by dissolving each 1ml oil with 100 ml of ethanol (99.5%, purity), which was subsequently mixed with the food by atomizing with the help of a spray bottle. Chemical analysis of the lavender essential oil was carried out by the Anadolu University Herbal Medicine and Scientific Research Center (Table 1). The basal diet used in the study was formulated according to the recommendations of NRC (1994). The contents and

Table 1 – Main components in Lavander oil (%).

Compounds	Relative %
1,8-cineole	1.20
(Z)-β-osimene	0.50
3-Octanone	1.10
Hexyl acetate	0.50
Octenyl acetate	1.10
Trans-Linalool oxide (Furanoid)	1.50
Cis-Linalool oxide (Furan)	0.90
Camphor	0.60
Linalool	31.60
Linalyl acetate	32.50
α-cantanel	0.60
β-caryophyllene	5.30
Terpinen-4-ol	3.90
(Z)-β-farnesene	5.10
Lavandulol	1.80
α-Terpineol	0.50
Borneol	1.00
Caryophyllene oxide	1.60
Total	91.30

nutrient content of the basal diet used in the study are presented in Table 2. The study consists of 4 groups. Diets with different levels of lavender essential oil added are as follows: 0 (control), 125, 250, 500mg/kg basal diet. The ambient temperature was gradually decreased from 33 °C in the first week to 22 °C on day 14, and was then kept constant afterwards. The lighting program applied was a continuous 23 h light.

Table 2 – Composition and nutrient concentrations of the basal diet.

Ingredients (%)	
Corn	62.93
Barley	1.00
Wheat bran, rough	1.00
Soybean meal, CP 48%	11.23
Cotton seed meal, CP 36%	10.00
Corn gluten, CP 62%	9.42
Dicalcium phosphate	1.83
Marble dust	1.25
L-Lysine hydrochloride	0.44
Salt	0.43
Vitamin-Mineral premix*	0.25
L-Threonine	0.12
DL-Methionine	0.10
Calculated values	
Dry matter,%	89.77
Crude protein,%	20.95
Ether extract, % Crude ash, %	2.14 6.06
Crude cellulose, %	3.45
Metabolic energy, kcal/kg	2.999
Methionine+Cystine,%	0.84
Lysine,%	1.09
Threonine,%	0.80
Tryptophan,%	1.09
Calcium,%	1.00
Available phosphorus, %	0.45

*Mineral-vitamin premix provided the following per kg of diet: retinol, 1.5 mg; dl-tocopherol, 12 mg; cholecalciferol, 0.1 mg; nicotinic acid, 15 mg; thiamin, 0.5 mg; pyridoxine, 0.9 mg; cyanocobalamin, 0.07 mg; menadione, 1 mg; folic acid, 0.25 mg; biotin, 0.025 mg; riboflavin, 2.1 mg; Fe, 50 mg; Cu, 6 mg; Mn, 60 mg; Zn,50 mg; Se, 0.2 mg; I, 1 mg; Co, 0.1 mg; CP: Crude Protein.

Determining performance values

The quails were weighed at the beginning of the study, and the weight at the beginning of fattening was determined as live weight. The quails and feed were then weighed on the 7th, 21st, and 35th days. At the end of the study, each replication was weighed and divided by the number of quails, the average of the repetitions was obtained, and the obtained values were used to calculate the final average group live weight. Feed consumption (FI) was calculated by subtracting the feeds given at the end of the experiment from the remaining feeds. The feed conversion ratio (FCR) was calculated by dividing the total feed consumed during the experiment by the difference between the final and initial body weights (live weight gain-BWG). At the end of the experiment, 20 individuals from each group (12 meat quality, 8 fatty acid analysis) were slaught.

Meat quality parameters

The samples (25 gram) of drumstick meat taken from quails were covered with strech film on polyethylene plates and stored at 4 ± 1 °C for further analysis (9 days). The pH and color parameters (L*(brightness), a*(redness), b*(yellowness)) of the samples were determined on the 1st, 3rd, 5th, 7th and 9th days. The pH values of the samples were obtained according to the method described by Gökalp *et al.* (2001). A solution with 10 g of homogenized samples was weighed in parallel and 100 ml of pure water was added. After homogenizing with an Ultra-Turrax device (T25, IKA Werk, Staufen, Germany) for 1 min, pH values were determined using a pH-meter (WTW Inolab, Weilheim, Germany). The color densities of the sectional surface of samples (L*, a*, b*) were detected using a Minolta colorimeter device (CR-200, Minolta Co, Osaka, Japan).

Microbial analysis of meat

Microbiological analyzes of meat samples were made according to the method by (Baumgart *et al.* 2015). Samples of drumstick meat were homogenized in 225 ml of sterilized Ringer solution. The dilutions in Ringer solutions were subsequently prepared. The pouring method was used in the inoculations for all bacteria. TMAB (Total number of mesophilic aerobic bacteria) was determined on Plate Count Agar (PCA, Merck, and Darmstadt, Germany) medium. The petri dishes were incubated aerobically at 30 ± 1 °C for 72 ± 1 h. TPAB (Total number of psychotrophilic aerobic bacteria) was determined on PCA medium. The petri dishes were incubated aerobically at 7 ± 1 °C for 9 days. The inoculation was performed by transferring 1 ml from the suitable dilutions with *Coliform spp*. counts into VRBA (Violet Red Bile Agar, Merck, and Darmstadt, Germany) medium. The petri plates were incubated in anaerobic conditions for 2 days at 30 °C. The *Micrococcus/Staphylococcus* number was determined on Mannitol Salt Agar (MSA, Merck, and Darmstadt, Germany) medium. Petri dishes in an aerobical medium were incubated at 30 ± 1 °C for 48 ± 1 h. The *Lactobacillus spp.* count was determined on MRS (de Man, Rogosa and Sharpe) Agar Base (Merck, Darmstadt, Germany) medium. The petri dishes were incubated anaerobically at 37 ± 1 °C for 72 ± 1 h.

The *Lactococcus spp.* number was determined on M17 Agar Base (Merck, Darmstadt, Germany) medium. The petri dishes were incubated aerobically at 37 ± 1 °C for 38 ± 1 h. The numbers of bacteria were expressed as log CFU/g

Lipid oxidation analysis of meat

In order to conduct the TBARS (Thiobarbituric Acid Reactive Substances) assay, which measures the existence of Malondialdehyde (MDA) in the sample, the homogeneous samples of meat (about 2 g) were homogenized with 12 ml of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% EDTA, 0.1% propyl gallate (dissolved in 3 ml of ethanol)) for 15–20 s in an Ultra-Turrax device (T25, IKA Werk, Staufen, Germany) and then filtered through a Whatman 1 filter paper. The filtrate (3 ml) was taken away to a test tube, 3 ml of thiobarbituric acid (TBA) (0.02 M) solution was added, and then it was homogenized again. Then, the test tubes were kept in a water bath for 40 min at 100 °C and subsequently cooled. After centrifugation (5 min at 2000 g), the absorbance values of the obtained liquid phase were obtained with the use of a spectrophotometer (AquaMate 7000 Vis Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) at 530 nm.

Homogenization and antioxidant analyses

Serum tissue separated from blood samples was taken at the end of the study, and CAT (catalog number: 201-24-0023), MDA (catalog number: 201-24-0037), and GSH (catalog number: 201-28-1357) levels were determined using a quail enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Sunred Biological Technology Co. Ltd, China), based on a double sandwich system. The kit prospectus was followed at every stage of the assay. The range of the MDA assay was 0.1 (nmol/mL) - 30 (nmol/mL), and the sensitivity was 0.096 (nmol/mL). The range of the GSH assay was 8 (mg/L) - 2000 (mg/L), and the sensitivity was 7.574 mg/L. The range of the CAT assay was 0.25 (ng/mL) - 70 (ng/mL), and the sensitivity was 0.237 ng/mL.

Fatty acid analysis of meat

Drumstick samples were homogenized with a paper shredder (Homogenizer HS-30E, witeg Labortechnik GmbH, Wertheim, Germany) using a polytetrafluoroethylene capped pestle (5553855 no, witeg Labortechnik GmbH, Wertheim, Germany). The grounded sample was mixed with 0.7 mL of potassium hydroxide (10 M) and 5.3 ml of methanol, and was then incubated at 55 °C for 45 min in an incubator (Nüve FN 120, Ankara, Turkey). 0.58 ml of H2SO4 (10 M) was added to the mixture, which was vortexed and incubated at 55 °C for 45 min again. 3 ml of n-hexane was subsequently added, and the tubes were centrifuged at 1600 g for 5 min (Nüve, Ankara, Turkey) (Wang *et al.*, 2015). After centrifugation, 1.5 ml of supernatant was put inside polytetrafluorethylene (PTFE)/ white silicone septa blue cap vials and later analyzed in a gas chromatography device (Thermo 1300, Thermo Fisher Scientific, Waltham, MA, USA) with an automatic sampler (Thermo AI 1310, Thermo Fisher Scientific, Waltham, MA, USA). In the analysis, a column of Fatty Acid Methyl Esters (FAME) (TR-FAME, cat no: P/N 260M154P, Thermo Fisher Scientific, Waltham, MA, USA) (length: 60 m, I.D.: 0.25 mm, film: 0.25 µm, and maximum temperature of 250/260 °C) was used. The initial temperature of the column was 100 °C; it was held for 3 min, then increased to 240 °C at a rate of 4 °C/min, and maintained for another 10 min. The device was operated in split mode, constant flow, 1 ml/min flow, 20 ml/min division, and 1:20 division ratio. Air flowswas worked at 350 ml/min flow and hydrogen 35 ml/min. The temperature of the FID (flame ionization detector) was 260 °C (Thermo AI 1310, Thermo Fisher Scientific, and Waltham, MA, USA). FAME mix (37C) standard solution (CL.40.13093.0001) in dichloromethane (Chem-Lab, Zedelgem, Belgium) was used for the identification of the peak. Helium was used as the carrier gas. Fatty acid identification was used by comparing and calculating the standard fatty acid peaks in the samples according to retention times using the Xcalibur program (Kramer *et al.*, 1997).

Intestinal Histomorphology

After slaughter, twenty-four samples of duodenum, jejunum and ileum from each main group (four flocks of six chickens per treatment) were kept in 10% neutral formalin solution for 48 hours to ensure their fixation. After the fixation process was completed, they were washed in tap water for 8 hours. The tissues were taken into cassettes, passed through a routine alcohol xylol series, and were then put in paraffin blocks. 5 µm sections of the blocks were taken on a slide and stained with hematoxylin-eosin. Then, the sections were examined considering each intestinal segment and all study groups. Villus length and villus thickness measurements were made using the NIH ImageJ software. While performing the examination, 5 areas were randomly selected from the intestinal segments. A total of 120 villi from 5 selected areas

were measured. Moreover, scoring for goblet cell was also performed. In the evaluation, the ratio of goblet cells to villus mucosa in the study groups was evaluated as less than 1% absent (0), 1-10% mild (1), 11-60% moderate (2), or more than 60% severe (3).

Enumeration of gut microbiota

At the end of the study, twenty-four quails from each main group (four pens of six broilers per treatment) were cut and their intestinal tracts were removed. For the isolation and enumeration of intestinal microorganisms, one gram of fecal content from each quail was aseptically collected and homogenized with 9 mL of 0.1% peptone water. Ten-fold dilutions were made in sterile peptone water from 10–1 to 10–6, and 0.1 ml from last three dilutions were plated in duplicate onto the relevant selective medium. *Escherichia coli* numbers were performed on Tryptone Bile X-Glucuronide (TBX) agar and incubated for 24 hours at 37 °C. *Enterococci* were cultured on Slanetz Bartley agar (SB, Oxoid CM377) and enumerated, followed by 24-48 hours of incubation at 37°C. *Enterobacteriaceae* and *Coliform spp.* were grown on Violet Red Bile Glucose agar (VRBG, Oxoid CM485) and Violet Red Bile agar (VRB, Oxoid CM107), using the pour plate technique and enumerated after 24-48 hours of incubated at 37 °C. *Lactobacillaceae* numbers were defined on MRS agar (Merck 110660). The plates were incubated for 24 h at 45°C under anaerobic conditions, and an anaerobic indicator (Mitsubishi) was included. Petri dishes containing 30 to 300 colonies were counted using a colony counter (Jin *et al.*, 1996). Microbial numbers were determined as log10 cfu per gram of fecal contents.

Statistical analysis

The data obtained were evaluated using SPSS 20.0 (IBM Corp., Armonk, NY, USA). A one-way analysis of variance (ANOVA) was conducted in order to determine whether there was a statistical difference between parameters and the relevant data. Duncan multiple comparison tests was used as post hoc tests for pairwise comparisons between groups. ($p < 0.05$). Villus length and thickness measurements were analyzed by ANOVA, the difference between groups in goblet cell analyzes was determined by Kruskal Wallis, which is one of the nonparametric tests, and the Mann Whitney U test was used for the group creating the difference. ($p<0.05$).

RESULTS

The current study investigates the effect of a lavender essential oil additive on performance parameters. In general, the live weight decreased in the lavender groups as compared to the control group ($p>0.05$). The feed consumption was lower on the third week as compared to the control group ($p<0.05$). Similarly, the feed utilization rate decreased in the lavender groups on the third week when compared to the control group ($p<0.05$) (Table 3).

This study investigates both the effect of the additive and of storage time. While the Lav500 group had a lower effect of time on pH levels on the first day,

Table 3 – Effects on growth performance parameters of the addition of lavender essential oil to quail diets at different doses (n = 50; mean ± standard error).

	Control	Lav125	Lav250	Lav500	
Times		Live weight			*p*
1.day, g	29.20±0.77b	31.40±0.52ab	31.00±0.24ab	31.87±0.54a	0.02
7. days, g	61.24±1.24	61.07±1.64	60.57±0.78	61.80±1.03	0.66
21. days, g	138.23±1.55	136.00±4.04	134.07±1.76	135.13±1.45	0.67
35. days, g	207.33±5.24	190.80±8.07	198.46±5.77	204.51±1.67	0.07
		Body weight gain			
1-7 days, g	32.04±1.40	29.68±1.31	29.57±0.68	29.93±1.07	0.41
8-21 days, g	76.99±1.31	74.93±3.09	73.50±1.02	73.33±0.52	0.46
22-35 days, g	69.11±4.37	54.80±4.81	64.39±5.31	69.37±2.32	0.10
		Feed intake			
1-7 days, g	70.65±1.27	74.20±1.22	73.23±1.75	75.10±2.07	0.61
8-21 days, g	130.31±3.79a	122.87±4.04ab	124.43±1.39ab	109.30±6.53b	0.02
22-35 days, g	262.96±13.25	240.58±10.68	253.97±10.14	263.87±5.63	0.25
		Feed conservation ratio			
1-7 days	1.15±0.02	1.22±0.02	1.21±0.04	1.22±0.02	0.60
8-21 days	0.94±0.03a	0.91±0.03ab	0.93±0.01ab	0.81±0.04b	0.04
22-35 days	1.27±0.04	1.26±0.02	1.28±0.02	1.29±0.02	0.19

*a-c – means with different superscripts in the same column are significantly different at $p<0.05$; g:gram.

pH levels were higher than the control group on the ninth day. The effect of the additive on the storage time in terms of the L meat color parameters value was lower on the 5th day compared to the control group (p<0.05), but higher on the 7th and 9th days (p<0.05). The effect of the additive on the storage time in terms of a* color parameters value was lower in Lav125 group on the seventh and ninth days as compared to the control group, but higher in the Lav250 and Lav500 groups (p<0.05). The effect of the additive on the storage time in terms of b* color parameter value was lower in the Lav125, Lav250 and Lav500 groups on the ninth day compared to the control group (p<0.05) (Table 4).

This study investigated the effect of the additive on the microbial load in meat and the storage time

Table 4 – Effects of storage time and diet on some meat parameters of quails fed diets supplemented with different doses of lavender essential oil.

Storage times (day)							
	Diets[1]	1	3	5	7	9	*p*
pH	Control	6.32±0.04a	6.13±0.09	6.39±0.14	6.34±0.02	6.37±0.01b	0.20
	Lav125	6.18±0.03ab	6.11±0.06	6.31±0.09	6.20±0.05	6.24±0.03c	0.20
	Lav250	6.05±0.01Cb	6.05±0.05C	6.21±0.05BC	6.32±0.10AB	6.46±0.02Aa	0.01
	Lav500	6.14±0.08Cab	6.04±0.02C	6.20±0.03BC	6.32±0.12AB	6.39±0.18Aab	0.04
	p	0.01	0.65	0.42	0.30	<0.001	
L	Control	43.17±1.77	43.06±0.36	47.74±1.06a	42.68±0.78b	44.35±1.02b	0.20
	Lav125	43.50±0.60C	41.35±1.33C	47.91±1.26Ba	49.24±1.05ABa	53.08±0.42Aa	<0.001
	Lav250	43.17±0.43	46.85±0.98	45.17±0.71a	45.56±0.96b	44.33±0.40b	0.37
	Lav500	46.81±0.60A	46.06±1.33A	40.91±1.26Bb	44.99±1.05Ab	45.69±0.42Ab	0.01
	p	0.07	0.39	0.03	0.02	<0.001	
a*	Control	14.92±0.81A	13.74±0.34A	11.33±0.90B	12.35±0.66ABab	12.89±0.23ABab	0.01
	Lav125	12.10±1.01	11.70±1.21	10.37±0.67	10.35±0.83b	12.06±0.45b	0.37
	Lav250	14.39±0.39A	10.88±0.35B	11.00±0.70B	12.76±0.46Aab	13.73±0.29Aa	<0.001
	Lav500	14.30±0.96	13.76±0.89	13.61±0.54	14.13±0.91a	14.08±0.25a	0.97
	p	0.13	0.12	0.07	0.03	<0.001	
b*	Control	6.63±1.09	7.71±1.09	8.12±0.33	9.20±1.47	11.38±0.44a	0.21
	Lav125	7.16±0.59B	8.79±0.31AB	8.08±0.87B	9.02±0.61AB	10.96±0.21Aab	<0.001
	Lav250	6.75±*0.15	8.66±0.72	7.35±0.33	9.70±0.30	9.85±0.20b	0.37
	Lav500	6.20±0.81B	6.87±0.98B	6.89±0.54B	9.57±0.86AB	10.97±0.21Aab	0.01
	p	0.80	0.88	0.39	0.94	0.01	

[1]Diets: C – basal diet, Lav125 – basal diet with 125 mg/kg lavender essential oil, Lav250– basal diet with 250 mg/kg lavender essential oil; Lav500- basal diet with 500 mg/kg lavender essential oil; L* – brightness, a* – redness, b* – yellowness, [a-c] – means with different superscripts in the same column are significantly different at p<0.05; A-C – means with different superscripts in the same row are significantly different at p<0.05.

(Table 3). We observed that TPAB values were lower in Lav125 group on the first and third days compared to the control group, and in the Lav250 and Lav500 groups on the ninth day (p<0.05); while TMAB values increased in Lav125, Lav250 and Lav500 groups on the first, fifth and ninth days compared to the control group (p<0.05). *Lactobasillus spp*. values increased in the Lav125 group on the third, fifth, seventh and ninth days compared to the control group (p>0.05); and also increased in the Lav125, Lav250 and Lav500 groups on the first, third and ninth days compared to the control group (p>0.05). Finally, *Enterococcus spp*. values decreased in the lavender groups on the seventh day as compared to the control group (p<0.05) (Table 5).

This study investigated the effect of the additive and storage on meat MDA (malondialdehyde) levels. We observed that MDA concentrations were lower in Lav250 and Lav500 groups on the fifth, seventh and ninth days compared to the control group (p<0.05) (Table 6).

There was a statistically significant difference in blood serum antioxidant enzyme concentrations between the control and lavender groups only in terms of MDA (p<0.05). There was no difference between the control and experimental groups in terms of catalase and GSH (p>0.05). MDA concentration decreased in the Lav125, Lav250 and Lav500 groups compared to the control group (p<0.05) (Table 7).

This study investigated the effect of the lavender additive on meat fatty acid profiles. Alpha linolenic acid (n-3), gamma linolenic acid (n-6), Arachidonic (n-6) and eicosenoic acid concentrations increased in the Lav125, Lav250 and Lav500 groups compared to the control group (p>0.05). The concentration of Eicosatrienoic acid (n-6) increased in the Lav125 group compared to the control group (p<0.05). The

Table 5 – Effect of storage time and diet on some bacterial counts in the meat of quails fed diets supplemented with different doses of lavender essential oil (log CFU/g), (n = 12).

Microbial load (log^{10} cfu)							
	Diets[1]	1	3	5	7	9	*p*
TPAB	Control	2.21±0.13Cab	2.41±0.09Ca	4.22±0.04B	5.63±0.60AB	6.40±0.17Aab	<0.001
	Lav125	1.65±0.05Db	1.93±0.03Db	3.29±0.29C	5.54±0.32B	6.93±0.04Aa	<0.001
	Lav250	2.00±0.001Bab	2.19±0.01Bab	4.05±0.23A	4.73±0.33A	5.54±0.28Abc	<0.001
	Lav500	2.39±0.16Ca	2.53±0.08Ca	3.78±0.12B	4.80±0.17A	5.21±0.05Ac	<0.001
	p	0.03	0.01	0.10	0.33	0.01	
TMAB	Control	3.88±0.18Cab	3.94±0.02C	4.22±0.06Bb	4.35±0.05AB	4.88±0.08Ab	<0.001
	Lav125	3.27±0.06Cb	3.82±0.28C	4.54±0.06Bab	4.67±0.31B	5.11±0.01Ab	0.01
	Lav250	4.35±0.06Ca	4.39±0.09C	4.96±0.02Ba	5.15±0.03B	5.71±0.06Aa	<0.001
	Lav500	4.07±0.01a	4.38±0.27	4.39±0.09b	4.45±0.25	5.13±0.12b	0.06
	p	0.01	0.22	<0.001	0.15	0.01	
Lactobacillus	Control	3.84±0.16	3.96±0.19	4.24±0.64	4.93±0.23	5.56±0.30	0.07
	Lav125	3.62±0.39B	4.51±0.28AB	5.12±0.06AB	5.29±0.03AB	5.64±0.49A	0.03
	Lav250	3.93±0.03B	4.39±0.18AB	4.64±0.34AB	4.90±0.29AB	5.21±0.05A	0.05
	Lav500	3.91±0.05	4.27±0.09	4.31±0.41	4.48±0.28	4.44±0.26	0.58
	p	0.74	0.35	0.50	0.24	0.09	
Lactococcus	Control	3.35±0.45B	3.99±0.09AB	4.22±0.06AB	4.53±0.12AB	4.92±0.06A	0.02
	Lav125	3.79±0.28B	4.64±0.30AB	4.69±0.39AB	5.32±0.09A	5.28±0.22A	0.03
	Lav250	3.31±0.01B	3.98±0.08B	5.06±0.25A	5.11±0.15A	5.11±0.07A	<0.001
	Lav500	4.16±0.65	4.56±0.22	4.59±0.36	4.58±0.40	5.04±0.04	0.55
	p	0.51	0.13	0.37	0.16	0.06	
Enterococcus	Control	2.66±0.10B	2.89±0.31AB	3.23±0.05AB	3.42±0.06ABa	3.61±0.04A	0.03
	Lav125	2.75±0.32	2.91±0.23	2.97±0.14	3.15±0.05ab	3.36±0.05	0.31
	Lav250	2.02±0.02B	2.66±0.36AB	2.85±0.01AB	2.99±0.02Ab	3.41±0.13A	0.02
	Lav500	2.73±0.21	3.17±0.02	3.24±0.16	3.37±0.09ab	4.07±0.50	0.09
	p	0.15	0.63	0.15	0.02	0.33	

[1]Diets: C – basal diet, Lav125 – basal diet with 125 mg/kg lavender essential oil, Lav250– basal diet with 250 mg/kg lavender essential oil; Lav500- basal diet with 500 mg/kg lavender essential oil; TMAB – total mesophilic aerobic bacteria count, TPAB – total psychrophilic bacteria count; [a-c] – means with different superscripts in the same column are significantly different at $p<0.05$; A-D – means with different superscripts in the same row are significantly different at $p<0.05$.

Table 6 – Effects of diet and storage time on MDA levels (µmol/kg) in the meat of quails fed diets supplemented with different doses of lavender essential oil.

	Malondialdehyde					
	Storaged days					
Diets[1]	1	3	5	7	9	p
Control	10.60±0.55D	10.44±0.22Dc	15.32±0.43Ca	19.69±0.11Aa	17.44±0.11Bb	<0.001
Lav125	10.81±0.20C	10.20±0.38Cc	14.17±0.31Bab	17.74±0.16Ab	18.94±0.53Aa	<0.001
Lav250	9.97±0.30C	13.16±0.30Ba	14.30±0.25ABab	13.72±0.49ABc	15.30±0.33Ac	<0.001
Lav500	11.19±0.39C	11.65±0.16Cb	13.55±0.30Bb	14.44±0.14Bc	15.88±0.26Ac	<0.001
p	0.20	<0.001	0.01	<0.001	<0.001	

[1]Diets: C – basal diet, Lav125 – basal diet with 125 mg/kg lavender essential oil, Lav250– basal diet with 250 mg/kg lavender essential oil; Lav500- basal diet with 500 mg/kg lavender essential oil; [a-c] – means with different superscripts in the same column are significantly different at $p<0.05$; A-D – means with different superscripts in the same row are significantly different at $p<0.05$.

concentration of docosahexaenoic acid (n-3) decreased in the Lav125, Lav250 and Lav500 groups compared to the control group ($p<0.05$). Oleic acid (n-9) concentration increased in the Lav500 group ($p<0.05$), while Palmitoleic acid concentration decreased in the lavender groups compared to the control group

Table 7 – Antioxidant enzyme concentrations in the blood serum of quails fed diets supplemented with lavender essential oil.

	Control	Lav125	Lav250	Lav500	*p*
Malondialdehyde, nmol/ml	5.21±0.30[a]	5.38±0.48[a]	3.39±0.79[ab]	2.20±0.99[b]	0.01
Catalase, ng/ml	6.77±0.64	9.17±0.58	6.58±0.56	7.46±0.45	0.89
Glutathione, mg/ml	276.33±26.55	213.31±27.44	190.67±22.04	247.21±26.11	0.69

[a,b] show the difference between groups ($p<0.05$).

(p<0.05). Palmitic acid concentration decreased in lavender groups compared to the control group (p>0.05). The saturated fatty acid (SFA) concentration decreased in the lavender groups compared to the control group (p<0.05). The unsaturated fatty acid (USFA) concentration increased in the lavender groups compared to the control group (p<0.05). The monounsaturated fatty acid (MUFA) concentration was lower in the Lav125 and Lav250 group, but it was similar to control in the Lav500 group (p<0.05). Polyunsaturated fatty acid (PUFA) concentration increased in the Lav125 and Lav250 groups (p<0.05). Omega-6 fatty acid (n-6) concentration increased in the Lav125 and Lav250 groups (p<0.05). Omega-9 fatty acid (n-9) concentration increased in the Lav500 group (p<0.05) (Table 8).

Table 8 – Effects of dietary addition of lavender essential oil on the fatty acid profile of quail drumstick meat, g/100 g (n = 8; mean ± standard error).

	Diet[1]				
	Control	Lav125	Lav250	Lav500	p
α-Linolenic Acid (C18:3n3) (ALA)	0.12±0.01^{b}	0.15±0.005^{b}	0.34±0.03^{a}	0.35±0.04^{a}	<0.001
Arachidonic Acid (C20:4n6) (AA)	0.05±0.001^{b}	0.12±0.01^{b}	0.38±0.06^{a}	0.42±0.04^{a}	<0.001
Beheric Acid (C22:0)	0.24±0.03^{b}	0.27±0.01^{b}	0.26±0.03^{b}	1.11±0.11^{a}	<0.001
Heptadecenoic Acid (C17:1)	0.17±0.01^{a}	0.10±0.03^{b}	0.18±0.05^{a}	0.13±0.02^{ab}	<0.001
Eicosatrienoic Acid (C20:3n3) (ETE)	0.06±0.01^{a}	0.05±0.004^{a}	0.05±0.005^{a}	0.01±0.01^{b}	0.57
Eicosenoic Acid (C20:1) (gondoic acid)	0.04±0.01^{c}	0.06±0.001^{c}	0.19±0.01^{b}	0.38±0.03^{a}	<0.001
Docosadienoic Acid (C22:2)	0.06±0.01^{ab}	0.02±0.005^{bc}	0.02±0.003^{bc}	0.04±0.005^{b}	0.02*
Eicosatrienoic Acid (C20:3n6) (DGLA)	6.47±0.48^{a}	7.48±0.85^{a}	5.60±0.54^{a}	1.13±0.06^{b}	<0.001
Docosahexaenoic Acid (C22:6n3) (DHA)	1.61±0.12	1.52±0.16	1.34±0.14	1.36±0.10	0.50
Eicosapentaenoic Acid (c20:5n3) (EPA)	0.55±0.02^{b}	0.72±0.05^{a}	0.40±0.02^{b}	0.47±0.02^{b}	0.03
γ-Linolenic Acid (C18:3n6) (GLA)	0.66±0.05^{b}	0.77±0.07^{ab}	0.85±0.06^{b}	1.01±0.11^{a}	0.04
Heneicosanoic Acid (C21:0)	0.14±0.01	0.12±0.01	0.14±0.01	0.16±0.02	0.39
Heptadecanoic Acid (C17:0)	0.17±0.01^{a}	0.10±0.001^{b}	0.18±0.005^{a}	0.13±0.02^{b}	<0.001
Lauric Acid (C12:0) (dodecanoate)	0.04±0.002^{b}	0.04±0.005^{ab}	0.04±0.001^{b}	0.06±0.005^{a}	0.01
Linoleic Acid (C18:2n6c)	22.04±0.88	23.29±0.60	24.32±1.10	21.73±0.35	0.17
Myristic Acid (C14:0)	0.53±0.06^{b}	0.64±0.05^{ab}	0.48±0.02^{b}	0.81±0.05^{a}	0.01
Myristoleic Acid (C14:1)	0.15±0.02a	0.14±0.01^{ab}	0.10±0.01^{b}	0.12±0.01^{ab}	0.05
Nervonic Acid (C24:1) (cis-15-tetracosenoate)	0.02±0.005c	0.03±0.005^{c}	0.15±0.02^{b}	0.33±0.03^{a}	<0.001
Oleic Acid (C18:1n9c)	32.17±1.21^{a}	27.23±0.88^{b}	30.26±0.94^{b}	34.10±0.51^{a}	<0.001
Palmitic Acid (C16:0)	21.65±0.65	21.32±0.25	20.28±0.12	20.41±0.52	0.12
Palmitoleic Acid (C16:1)	6.39±0.69^{a}	4.23±0.37^{b}	3.77±0.45^{b}	4.60±0.17^{ab}	0.01
Pentadecanoic Acid (C15:0)	0.16±0.01^{b}	0.14±0.03^{b}	0.16±0.02^{b}	0.36±0.05^{a}	<0.001
Stearic Acid (C18:0)	10.88±0.78^{b}	11.23±0.33^{b}	12.09±0.72^{ab}	14.65±0.61^{a}	0.02
Tricosanoic Acid (C23:0)	0.15±0.04^{b}	0.15±0.02^{b}	0.31±0.01^{ab}	0.32±0.06^{a}	0.02
ΣSFA	41.82±0.43^{a}	33.93±0.64^{b}	33.23±1.01^{b}	36.60±1.62^{ab}	0.02
ΣUSFA	55.38±2.42^{b}	65.18±1.08^{a}	65.88±0.61^{a}	65.26±1.45^{a}	<0.001
ΣMUFA	38.56±0.88^{a}	32.73±1.20^{b}	34.50±0.97^{ab}	38.56±0.73^{a}	<0.001
ΣPUFA	31.36±0.85^{a}	33.99±0.36^{a}	33.29±0.82^{a}	26.02±1.08^{b}	<0.001
Σn-3	2.58±0.26	2.37±0.21	2.18±0.17	2.09±0.17	0.43
Σn-6	29.02±1.07^{a}	31.96±0.30^{a}	31.08±0.73^{a}	24.15±1.26^{b}	<0.001
Σn-9	37.10±1.53^{ab}	32.69±1.19^{b}	35.89±0.75^{ab}	39.71±0.57^{a}	<0.001
n-3/n-6	0.11±0.02^{a}	0.07±0.01^{ab}	0.07±0.001^{ab}	0.06±0.01^{b}	<0.04
MCFA	0.06±0.001^{b}	0.05±0.01^{b}	0.13±0.02^{ab}	0.11±0.01^{a}	<0.001
LCFA	97.87±0.14	97.08±0.47	97.60±0.30	96.36±0.40	0.10
VLCFA	2.51±0.14	2.10±0.07	2.29±0.22	2.67±0.28	0.26

[1]Diets: C – basal diet, Lav125 – basal diet with 125 mg/kg lavender essential oil, Lav250– basal diet with 250 mg/kg lavender essential oil; Lav500- basal diet with 500 mg/kg lavender essential oil; ΣSFA – total saturated fatty acids, ΣUFA – total unsaturated fatty acids, ΣMUFA – total monounsaturated fatty acids, ΣPUFA – total polyunsaturated fatty acids, Σn-3 – total omega 3 fatty acids, Σn-6 – total omega 6 fatty acids, Σn-9 – total omega 9 fatty acids, n-3/n-6 – ratio of omega-3 and omega-6 fatty acids, MCFA – medium-chain fatty acids, LCFA – long-chain fatty acids, VLCFA – very long-chain fatty acids; $^{a-c}$ – means with different superscripts in the same row are significantly different at p<0.05.

This study investigated the effect of the additive on gut microbiota counts. *Lactobacillus spp.* count increased in the Lav125 and Lav250 groups compared to the control group (p<0.05). *Enterobacteriaceae* count decreased in the Lav250 group compared to the control group (p<0.05). *Escherichia coli* count increased in the Lav125, Lav250 and Lav500 groups compared to the control group (p<0.05) (Table 9).

Table 9 – Effect of Lavender essential oil added to quail diets on gut microbiota (gram/Log10).

	Control	Lav125	Lav250	Lav500	*p*
Lactobacillus spp.	5.64±0.11^{c}	6.11±0.06^{b}	6.32±0.08^{b}	7.20±0.08^{a}	<0.001
Coliform spp.	7.44±0.09	7.41±0.16	7.39±0.10	7.27±0.05	0.64
Enterobactericeae	7.45±0.11^{a}	7.13±0.11^{ab}	6.88±0.10^{b}	7.25±0.05^{ab}	<0.001
Escherchia coli	7.66±0.05^{a}	7.36±0.07^{b}	7.51±0.07^{ab}	7.37±0.08^{ab}	0.02
Enterococci spp.	6.69±0.01	6.42±0.12	6.66±0.14	6.63±0.13	0.35

[1]Diets: C – basal diet, Lav125 – basal diet with 125 mg/kg lavender essential oil, Lav250– basal diet with 250 mg/kg lavender essential oil; Lav500- basal diet with 500 mg/kg lavender essential oil; $^{a-c}$ – means with different superscripts in the same column are significantly different at $p<0.05$.

There was no significant difference in the length and thickness of the villi in the duodenum in the Lav125 and Lav250 groups compared to the control group, while both length and thickness increased in the Lav500 group. When the jejunum segment was examined, there was no significant difference in terms of villus length in the Lav125 and Lav500 groups compared to the control group, while villus length was found to be shorter in the Lav250 group compared to the other groups. Villus thicknesses increased in the Lav125, Lav250 and Lav500 groups compared to the control group. In ileum samples, while villus lengths were close in the control and Lav500 groups, they decreased in the Lav250 group and increased in the Lav500 group. While no difference was observed in the control, Lav125 and Lav500 groups in terms of villus thickness, it was observed that it decreased in the Lav250 group compared to the control group (Table 10). There was a mild increase in the duodenum goblet score in the control and Lav250 groups, while the increase was moderate in the Lav125 and Lav500 groups. Similarly, jejunum goblet cell score increases were in the control and Lav250 groups, moderate in the Lav125 group, and severe in the Lav500 group. In ileum samples, no difference was observed between the groups, while the score increase of goblet cells was generally mild (Figure 1).

Table 10 – Effect of adding lavender essential oil to quail diets on the villus length and thickness per intestinal segment.

		Control	Lav125	Lav250	Lav500
Duodenum	Villus length	97.00±3.16^{a}	94.00±1.41^{b}	90.00±3.16^{b}	109.00±2.00^{c}
	Villus thickness	16.00±1.41^{a}	18.00±2.28^{a}	18.00±1.41^{a}	30.00±2.83^{b}
Jejunum	Villus length	72.00±3.16^{a}	74.00±0.89^{a}	63.00±2.82^{b}	77.00±2.68^{c}
	Villus thickness	9.00±0.63^{a}	20.00±1.41^{b}	15.00±2.00^{c}	16.00±2.28^{c}
Ileum	Villus length	61.00±1.41^{a}	70.00±3.69^{b}	34.00±0.89^{c}	58.00±1.26^{a}
	Villus thickness	13.00±1.41^{a}	16.00±2.28^{a}	8.00±0.63^{b}	18.00±2.83^{c}

$^{a, b, c}$ show the difference between the same lines. (Mean+SD, µm).

DISCUSSION

Effect on fattening performance

Lavender essential oil can be found in linalol, linalyl acetate and some other mono-and sesquiterpenes, flavonoids such as luteolin, triterpenoids such as ursolic acid, coumarins such as umbelliferon and coumarin, and the leaves and flowers of the lavender plant (Renaud *et al.*, 2001; Adaszyńska-Skwirzyńska and Szczerbińska, 2018a). The active compounds in plant oils are likely to stabilize the intestinal microbial flora and stimulate the secretion of endogenous digestive enzymes, thereby improving growth performance in poultry (Cross *et al.*, 2007; Brenes & Roura, 2010). In the current study, we did not observe a significant decrease in terms of live weight in the lavender groups compared to the control group. It can be said that

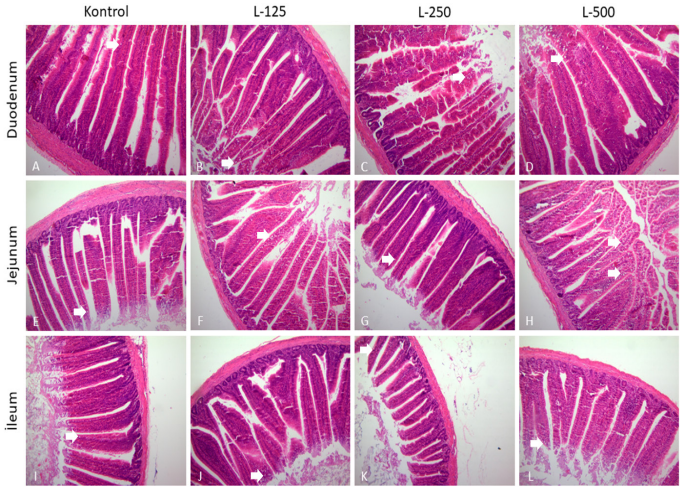

Figure 1 – The effect of lavender essential oil addition to diet on ileum, caecum, and colon tissues.

A. Control Group. Slight goblet cell (arrow). B. Lav125 Group. Intermediate goblet cell (arrow). C. Lav250 Group. Slight goblet cell (arrow). D. Lav500 Group. Intermediate goblet cell (arrow). E. Control Group. Slight goblet cell (arrow). F. Lav125 Group. Intermediate goblet cell (arrow). G. Lav250 Group. Slight goblet cell (arrow). H. Lav500 Group. Severe goblet cell (arrows). I. Control Group. Slight goblet cell (arrow). J. Lav125 Group. Slight goblet cell (arrow). K. Lav250 Group. Slight goblet cell (arrow). L. Lav500 Group. Slight goblet cell (arrow).

the high daily live weight gain in the Lav500 group between the 22nd and 35th day and the decrease in feed consumption and feed utilization rate in the Lav500 group between the 8th and 21st day are positive features caused by lavender supplementation. Several previous studies using herbal extracts obtained from the Labiatae family have reported no beneficial effects on performance parameters for the animals (Botsoglou *et al.*, 2002; Hernandez *et al.*, 2004; Reisinger *et al.*, 2011; Akbarian *et al.*, 2013). However, extracts from plants belonging to the Labiatae family, such as thyme (Giannenas *et al.*, 2005; Bampidis *et al.*, 2005) and rosemary (Spernakova *et al.*, 2007), have also been reported to stimulate growth performance when used in broiler diets. Salajegheh *et al.* (2018) concluded that lavender powder (1%) significantly increases FI during the fattening period. Adaszyńska-Skwirzyńska & Szczerbińska (2018a) reported an increase in live weight and, in turn, an improvement in the utilization rate of feed for production (BW, FCR) upon the addition of lavender essential oil at a higher concentration (0.4 mL/L). Adding 400 ppm of lavender essential oil to the diet did not have any negative effects on FI in broilers (Salarmoini *et al.*, 2019). In general, the variability in the effectiveness of phytogenic feed additives on quail performance parameters (live weight, feed consumption and feed preservation rate) may vary depending on the animal, diet composition, plant extraction method, harvest time and storage period.

Effect on pH parameter in meat

Meat pH can be affected by a large number of factors, such as age, gender, feed additives, stress before slaughter or hormonal status, muscle morphology and glycogen content. A high pH value in meat is associated with dark-colored meat, while lower pH values are associated with lighter-colored meat (Fletcher, 1989). Regarding the effect of storage on meat quality in the present study, meat pH increased with the progression of time in the Lav250 and Lav500 groups. Moreover, pH levels were higher in the Lav250 and Lav500 groups compared to the control group on the ninth day. In current study, quail meat pH values were within the range reported by similar studies (Genchev *et al.*, 2010; Özbilgin *et al.*, 2021, 2022). The oxidation products of unsaturated fats are acidic molecules such as hydroxyl acid, keto acid, and smaller fatty acid molecules (Belitz *et al.*, 2004). The increase in meat pH can be attributed to the antioxidant effect of lavender essential oil on the oxidation of unsaturated fatty acids, which causes the production of acidic molecules.

Effect on quality parameters in meat

Sensory elements such as color and smell are important parameters in consumer market preferences. There are studies which report that the color of meat is easily affected by sex, age, species, fiber composition, diet and environmental factors (Te Pas *et al.*, 2004; Aksu *et al.*, 2011; Özbilgin *et al.*, 2021). Changes in meat color are due to the oxidation of oxymyoglobin into methemoglobin, which turns the red meat color into brown (Nerín *et al.*, 2006). In the current study, the a* value showed a significant increase in the lavender groups compared to the control group, especially in the Lav250 group, due to the effect of storage time. Similarly, it was observed that the b* value increased in the Lav125 and Lav500 groups depending on the storage time. In general, the storage time and the addition of lavender essential oil resulted in an increase in the L* and a* values and a decrease in the b* value. Also, lavender essential oil supplementation in this study caused an increase in the L* value in the Lav125 group from the first day to the ninth day, while it caused a decrease in the Lav500 group during the same period. Some studies show that natural antioxidants can delay the darkening of meat color by delaying the formation of methemoglobin, prolonging the color a *. a* and b* meat colors were at a higher concentration in lambs fed with dietary supplementation of thyme essential oil (1 ml thyme essential oil / kg of feed), one of the natural antioxidants that affect meat color (Simitzis *et al.*, 2008). Soares *et al.* (2003) reported that a significant increase in the L* value in poultry meat is closely related to its total antioxidant capacity due to the relationship between the brightness of the meat (L* value) and the activity of phospholipase A2, an enzyme that oxidizes phospholipids in meat. Jang *et al.* (2008) reported that the L value decreased in broilers in a study in which they added medicinal plant extracts to the diet. Küçükyılmaz *et al.* (2017) reported results like the current study, with the supplementation of lavender essential oil (24-48mg/kg feed) to broiler diets producing an increase in the L* value in meat compared to the control group. Simitzis *et al.* (2008) explained that supplementing thyme essential oil to the diet indirectly changes the color of meat by reducing hemoglobin oxidation and activating mechanisms that change pigment distribution in animal tissues. In general, in the current study, it was observed that adding lavender essential oil increases the L value in a dose-dependent manner.

Effect on microbial load in meat

The antimicrobial activity of essential oils is due to the presence of secondary metabolites. The hydrophobic components contained in these metabolites interact with lipids present in the cell membrane of microorganisms (da Silva *et al.*, 2021). This interaction causes the loss of membrane integrity of microorganisms that cause spoilage. This damage then causes changes to the functioning of the electron transport chain, absorption of nutrients, coagulation of cellular contents, both protein and nucleic acid synthesis, and inhibition of enzymes. It has been reported that aromatic phytochemicals (and especially essential oils) can be used against lipid oxidation in meat and its products (Bozin *et al.*, 2007; Govaris *et al.*, 2010). Bacteria that develop on meat at cold temperatures (7°C and below) are considered psychrotrophic. They consist of both gram-positive bacteria like lactic acid bacteria and negative bacteria such as *Pseudomonas spp*. and *Enterobacteriaceae* (Gill & Newton 1978; Holzapfel, 1998). *Pseudomonas* spp. play a role in the spoilage of stored meat, especially at cold temperatures (Ercolini *et al.*, 2007; Jay *et al.*, 2003; Labadie, 1999). In particular, the microflora of vacuum-packed cold-stored meat contains lactic acid bacteria in most cases (Borch *et al.*, 1996; Dainty *et al.*, 1983; Hitchener *et al.*, 1982; Nychas *et al.*, 1998; Shaw *et al.*, 1984). The number of TPAB on the ninth day of this study decreased according to the dosage in lavender groups compared to the control group. This can be attributed to the effect of lavender essential oil. Total mesophilic bacteria numbers in poultry are an indication of the level of hygiene. The total number of coliforms and the total number of fecal coliforms are indicators of environmental contamination. On the other hand, *Staphylococcus aureus* numbers are an indicative of poor hygiene, transportation and temperature control status (González-Fandos & Dominguez, 2006; Rindhe *et al.*, 2008). In the current study, the TMAB number was not affected by the addition of lavender essential oil compared to the control group. Since there was no difference between the lavender supplement and the control group, it is believed that the meat was not contaminated. In previous research, it was reported that essential oils such as mustard, thyme, oregano, cinnamon, and garlic, and components such as thymol, carvacrol, and cinnamaldehyde present broad-spectrum antimicrobial activity against foodborne pathogens, including *E. coli* (Clemente *et al.*, 2016; Yuan *et al*, 2019). Similarly, Ouattara *et al.* (1997) and Gutierrez *et al.* (2009) observed the inhibitory effects of these oils against *E. coli* and *Salmonella spp*. It was reported that treating chicken meat with thyme essential oil significantly inhibits the increase of lactic acid bacteria (LAB), despite the prolongation of storage times. Dzudie *et al.* (2004) found that adding ginger or basil essential oils to beef meatballs significantly reduced LAB and *E. coli* compared to the control group, ensuring food security. Mastromatteo *et al.* (2009) reported that thymol and carvacrol reduced the cell load of LAB and Enterobacteriaceae, although the storage time in chicken meatballs increased with the counts. Similar to previous studies, in the current study, the LAB number decreased in the lavender groups on the ninth day compared to the control group, depending on the storage time.

Effect on lipid peroxidation in meat

The most obvious effect of adding essential oils to poultry diets is on meat lipid oxidation, since white meat has a high concentration of lipids. In the literature, it is reported that the addition of essential oil reduces the concentration of malondialdehyde (MDA) depending on the storage time (Aksu *et al.*, 2014; Salarmoini *et al.*, 2019). In previous studies, similar to the current study, it was reported that adding varying concentrations of essential oils leads to a decrease in MDA concentration during the storage process in broilers consuming the diets with added *Lavandula angustifolia* (100-400mg/kg feed, Salarmoini *et al.* 2019) and *Lavandula stocheas* (0-48mg/kg feed, Küçükyılmaz *et al.*, 2017), Japanese quails consuming the diets with added oregano oil (200-600mg/kg feed, Önel & Aksu, 2019), and meatballs sprayed with cinnamon extract (200 mg, Chan *et al.*, 2014). In the current study, although the storage period was prolonged, the dose-dependent decrease in MDA concentration in lavender groups on the fifth, the seventh and the ninth days can be attributed to the lavender effect.

Effect on antioxidant enzymes

There are studies of many researchers on antioxidant properties stating that essential oils obtained from plants are useful in delaying lipid peroxidation in diets (Amer *et al.*, 2022a, 2022b, Omar *et al.*, 2022, Imbabi *et al.*, 2021). In the current study, MDA concentration in the blood serum due to lipid peroxidation is expected to decrease in the lavender groups compared to the control group. Phenolic compounds suggest an important role of essential oils as antioxidants. There has been a study reporting that lavender essential oil added to the diet decreased MDA levels in the blood

serum like in the current study (Barberastani *et al.*, 2020). In the current study, the addition of lavender essential oil to the diet increased CAT serum enzyme activity in the Lav125 group compared to the control group. Adding essential oils to quail diets can increase the oxidative stability of tissues by adding natural antioxidants (Barbarestani *et al.*, 2020). In addition, the antioxidant activity of lavender essential oil can be attributed to its content of terpenoids such as α-pinene, terpineol, eucalyptol and fellandral (Carrasco *et al.*, 2016).

Effect on fatty acid profile in meat

The fatty acid profile of chicken meat is affected by nutrient contents and genetic factors (Gou *et al.*, 2020; Zanetti *et al.*, 2010). Chicken meat is richer in PUFA than other meats because the diet of broilers is usually rich in PUFA (Smet *et al.*, 2008). Recently, dietary manipulations seeking to change meat fatty acid profiles have been a popular research topic (Amer *et al.*, 2021; Kishawy *et al.*, 2019; Giannenas *et al.*, 2018; Saleh *et al.*, 2018). The use of plant extracts in many areas, as well as their potential to increase production capacity and improve poultry health, have been reported the main purpose of enriching poultry diets with plant extracts (Dhama *et al.*, 2015). In the current study, with the addition of lavender essential oil to the diet, the concentration of PUFA was especially high in the Lav125 and Lav250 groups compared to the control group. The addition of lavender essential oil to the diet significantly reduced the concentration of SFA compared to the control group. Moreover, adding lavender essential oil to the diet increased the n-6 PUFA (especially gamma-linolenic acids, mainly linoleic acid and arachidonic acid) concentration in meat and the oleic acid (n-9) concentration in the Lav500 group compared to the control group, depending on the increase on the dosage of lavender. Kartikasari *et al.* (2012) observed that broiler rations with added short-chain n-3 PUFA (-linolenic acid) led to an increase in the concentration of long-chain n-3 PUFA (eicosapentaenoic acid (20:5)) concentration. In the current study, it was found that the addition of lavender essential oil to the diet increases the concentration of n-3 PUFA (EPA and DHA).

Effect on intestinal histomorphology

Villi and crypts are two important components of the small intestine, and their geometry is an indicator of the absorptive capacity of the small intestine (Heydarian *et al.*, 2020). The turnover of the intestinal epithelium provides the dynamic balance (Su *et al.*, 2018). In the current study, villus heights in the duodenum and jejunum were significantly increased in the Lav500 group compared to the control group. Hashemipour *et al.* (2013) reported that villi were higher when 100 or 200 ppm of carvacrol and thymol (the main compound of the thyme plant) were added to broiler diets. In general, polyphenolic compounds can increase villus height and villus height, since they can increase the absorption surface area, and the efficiency of digestion and absorption of nutrients (Reisinger *et al.* 2011; Hong *et al.* 2012; Khattak *et al.* 2014). Similar to the current study, Salarmoini *et al.* (2019) reported that lavender supplementation in broiler diets significantly decreased crypt depth and increased villus height compared to control group at all lavender levels.

Effect on gut microbiota

Intestinal microflora is an important factor for animal health and yield characteristics, such as meat and milk. However, it is also very important for human health, as the carcass can be contaminated with various pathogens that live in the intestinal flora, such as *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Clostridium perfringens, Campylobacter spp.* and *Salmonella spp.* (Choi *et al.*, 2015). The intestinal microflora in poultry is qualitatively and quantitatively influenced by many factors such as environmental stress, farm conditions, the age of the animal, and the composition of the feed. The homeostasis of the gastrointestinal flora can be regulated by certain active substances, such as essential oils introduced into the diet (Roberts *et al.*, 2015).

Another study investigating the effect of different essential oils on bacterial colonization in various parts of the poultry intestine discovered that the addition of essential oils decreased the number of *Escherichia coli, Clostridium perfringens* and *Enterococcus spp., Salmonella spp.,* and *Staphylococcus spp.* colonies (Cross *et al.*, 2007; Tiihonen *et al.*, 2010; Kırkpınar *et al.*, 2011; Erhan *et al.*, 2012; Hong *et al.*, 2012; Vuki´c -Vranje˘S *et al.*, 2013). Moreover, the addition of pennyroyal essential oil (Mentha pulegium L.) to the feed at the proportions of 0.25% and 0.5%, was reported to have a positive effect on the number of LAB (Erhan *et al.*, 2012). There are also studies which report that it has inhibitory effects against the reproduction of *Lactobacillus spp*. (Tiihonen *et al.*, 2010; Hong *et al.*, 2012). It is important to select appropriate biological active substances that will reduce the number of

enteric pathogens without affecting LAB (Choct, 2009). Adaszyńska-Skwirzyńska and Szczerbińska (2018b) found that, while the addition of lavender essential oil to the drinking water of broiler chickens decreased the number of pathogenic microorganisms (*Escherichia coli* and *Coliform spp.*), it increased probiotic bacteria in the intestinal microflora of the ileum. However, Mokhtari *et al.* (2018) discovered that *E. coli, Coliform spp.* and *Lactobacillus spp.* numbers were lower in the secum flora of broilers that consumed a ration with the addition of 600 mg/kg lavender when compared to other groups. As a result, they reported that lavender and some herbs exhibit antibacterial activity. In the current study, lavender essential oil added to quail diets was observed to decrease the CFU of *E. coli* and *Coliform spp.* bacteria, and to increase the number of *Lactobacillus spp*. colonies (probiotic bacteria), similar to many previous studies ($p<0.05$). As a result, due to its antimicrobial effect against *E. coli* and many pathogenic bacteria, it is believed that lavender essential oil in the right concentrations can be a good dietary supplement for poultry feed. The antimicrobial effect is thought to be mainly due to the potential of essential oils to enter the bacterial cell membrane, break down membrane structures, and cause ion outflow. In addition, it was reported that the active compounds contained in essential oil extracts stimulate intestinal mucus secretion in broiler chickens and, accordingly, prevent pathogens from attaching to the intestinal wall (Jamroz *et al.*, 2006).

CONCLUSION

When adding lavender essential oil to quail diets, the Lav500 group had the highest body weight change after the control group. In terms of feed conservation rate and feed intake, the Lav500 group had the lowest consumption as compared to the control group between the eighth and twenty first days of the study. Additionally, it was observed in the current study that storage periods can cause changes in the color of meat or meat products. In terms of the effect of storage on meat quality in this study, meat pH increased with the progression of time in the Lav250 and Lav500 groups. Additionally, it was observed that the L* and a* values increased positively due to the effect of storage time upon the addition of lavender compared to the control group. The addition of lavender essential oil to quail diets increased the concentrations of n-6 PUFA (gamma-linolenic acid and linoleic acid), n-3 PUFA (EPA and DHA), and n-9 PUFA (oleic acid). In general, regarding the antioxidant effect of the lavender plant, it can be said that phenols (camphor in this study) can be preferred as a more effective substance against lipid peroxidation. It is believed that, due to its antimicrobial effect against pathogenic bacteria such as *E. coli*, essential oils can be a good dietary supplement for poultry feed. Moreover, in the current study, villi height and resorption in the duodenum and jejunum were significantly increased in the Lav500 group as compared to the control group. The concentration of MDA, a blood serum antioxidant enzyme, decreased with the addition of lavender oil. Also, lavender essential oil added to quail diets was observed to increase the number of *Lactobacillus spp.* (probiotic bacteria) colonies. As a result, it is thought that continuing studies on adding lavender oil to poultry rations will have important consequences for poultry production.

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CONFLICT OF INTEREST

The authors declare no competing interests

ETHICAL APPROVAL

This study has been conducted with the permission of Sivas Cumhuriyet University, Animal Experiments Local Ethics Committee dated 08.04.2021 and numbered 422.

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