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Augmenting broiler diets with essential oils affects growth, intestinal microbiota and morphology, and meat quality

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Abstract

The health and productivity of broiler chickens are related to their intestinal microbiota, which may be influenced by supplemented feed components. This trial intended to evaluate the effects of a dietary mixture of essential oils from oregano (*Origanum vulgare ssp.* hirtum), sage (*Salvia triloba* L.), and lavender (*Lavandula angustifolia* L.) on broiler chicken growth performance, intestinal microbiota, intestinal morphology, and meat chemical composition and oxidative stability. A total of 288 one-day-old male Ross-308 chicks were randomly assigned to four treatments with six replicate pens (12 chicks per pen). The chicks of the control treatment were fed typical commercial maize and soybean meal rations in mash form. The rations of the other three treatments were supplemented with a mixture of essential oils at 100, 200, and 400 mg/kg, respectively. At the end of the trial (day 42), tissue samples were collected for analysis. Major bioactive components of the three essential oils were identified by gas chromatography-mass spectrometry. Essential oil supplementation increased the radical scavenging capacity and the total phenolic content of the feeds. Performance parameters (weight gain, feed intake, feed conversion ratio, European production efficiency factor) were not affected significantly by the supplementation. Intestinal microflora populations (determined by matrix-assisted laser desorption ionization-time of flight mass spectrometry) were modified significantly in both the cecum and the jejunum. Breast and thigh meat oxidative stability under refrigerated storage was improved significantly. Additional research is required to elucidate the potential synergistic effects of dietary mixtures of essential oils.

Keywords: bioactive compounds, chicken, intestinal architecture, intestinal microflora, MALDI-TOF mass spectrometry, oxidative stability

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Introduction

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Broiler productivity has been founded on rearing industry systems that focus on disease prevention and medication. The administration of antibiotics as a therapeutic agent has been one of the most popular practices to not only control disease, but stimulate growth in broilers (Huyghebaert *et al.*, 2011; Valenzuela-Grijalva *et al.*, 2017). Although antibiotic substances and their metabolites could hinder the growth of microorganisms, even in low dosages, their use as antibiotic growth promoters (AGPs) to enhance feed efficiency has raised health issues related to their ability to accelerate bacterial resistance as a result of uncontrolled use (Huyghebaert *et al.*, 2011). Thus, the use of AGPs as zootechnical additives in poultry diets has been restricted worldwide, and it has been banned since 2006 in European Union (EU) countries (Christaki *et al.*, 2012). Therefore, the application of the current legislation has led the research community to innovate natural, safer, and more easily accessible products that could replace AGPs. Such compounds include spices, herbs, plant extracts, antioxidants, enzymes, probiotics, and prebiotics (Christaki *et al.*, 2012; Zeng *et al.*, 2015; Valenzuela-Grijalva *et al.*, 2017; Cimrin *et al.*, 2020; Sugiharto *et al.*, 2021).

Currently, consumer awareness of the risks and implications of the application of medication in animal feed, nutritional, and overall health issues has led to a demand for functional and drug-free food products (Voidarou *et al.*, 2007). Thus, in recent years many research studies have been performed, aiming to enrich meat and animal fats with beneficial components to improve meat quality and to create functional foods that may attenuate serious metabolic disorders (Petracci *et al.*, 2013) and even ageing (Wilson *et al.*, 2017).

The natural phyto-additives in chickens that have been studied the most are extracts and essential oils (EOs) from plants of the Lamiaceae family (Christaki *et al.*, 2012; Zeng *et al.*, 2015; Sevim *et al.*, 2020). Dried plant material and EOs of oregano (*Origanum vulgare ssp.* hirtum), sage (*Salvia triloba L.*), and lavender (*Lavandula angustifolia L.*) have been utilized since antiquity for therapeutic and cosmetic reasons (Christaki *et al.*, 2012; Zeng *et al.*, 2015). Phytogenic products from these aromatic plants contain phenols and polyphenols as the main components, which have antimicrobial, antioxidant, anti-coccidial, immunomodulatory, and digestion-enhancing properties (Bozkurt *et al.*, 2013; Ullah *et al.*, 2020). Furthermore, the EOs of oregano, rosemary, and sage can be utilized to reduce the oxidative damage of poultry meat and improve its chemical and sensory traits (Christaki *et al.*, 2012; Franciosini *et al.*, 2016; Sevim *et al.*, 2020).

However, little is known about the specific modes of action of sage and lavender and the possible interactions of their combined use with oregano. To the authors' knowledge, there are no scientific publications about the combined dietary use of oregano, sage, and lavender on broiler performance, composition of intestinal microflora, or the antioxidant status of chicken meat. Therefore, in the present study, these parameters were assessed after in-feed combined use of these EOs at incremental concentrations in broiler chickens reared up to 42 days old.

Materials and Methods

This trial was conducted according to the regulations of local veterinary services (Presidential Decrees, 2013) and the authorities of the School of Agriculture of University of Ioannina, Greece (UOI University Research Committee Research Registration: 61300).

Three herbal EOs were examined from three plants, namely i) oregano (*Origanum vulgare* ssp. hirtum), ii) sage (*Salvia triloba L.*), and iii) lavender (*Lavandula angustifolia L.*) These EOs were procured from HerbsHellas SA, Chloi, Velestino Magnesia, Greece.

For this trial, 288 one-day-old male Ross-308 chicks (initial body weight 38.5 ± 0.56 g) were acquired from PINDOS APSI hatchery and reared at a commercial poultry farm in Arta, Epirus, Greece. Each of the four treatments involved six replicate pens (1.2 m^2) of 12 chicks each. The chicks were reared under commercial breeding and management practices in controlled ambient temperature and humidity conditions. All chicks were vaccinated against Newcastle disease, infectious bronchitis, and infectious bursal disease (Gumboro) at the hatchery. Feed and drinking water were given to the chicks *ad libitum*.

The birds of the control treatment (ESOIL0) were fed typical commercial rations in mash form, based on maize and soybean meal (Table 1) and were formulated to satisfy breeder recommendations (Aviagen, 2019) using the ingredient matrix data from the databases of Premier Nutrition (2014) and the NRC (1994). The other three treatments were fed the same rations supplemented with EOs. Treatment ESOIL100 was supplemented with EOs at 100 mg/kg of feed (50 mg oregano EO; 25 mg sage EO; 25 mg lavender EO). Treatment ESOIL200 was supplemented with EOs at 200 mg/kg of feed (100 mg oregano EO; 50 mg sage EO; 50 mg lavender EO). Treatment ESOIL400 was supplemented with EOs at 400 mg/kg of feed (200 mg oregano EO; 100 mg sage EO; and 100 mg lavender EO).

The birds were weighed on days 1, 14, 28, and 42. Feed intake and mortality were recorded daily. Feed conversion ratio (FCR) was evaluated as the ratio of feed intake to body weight gain. The European production efficiency factor (EPEF) was calculated for the overall trial (KPI Library, 2021) using the formula:

EPEF = [average daily weight gain (g) × survival rate (%)] / [feed conversion ratio × 10]

On day 42, all chickens were slaughtered under commercial conditions. From each pen, four birds were chosen at random for meat analysis and four for intestinal architecture and microbiological analysis.

Table 1 Broiler chicken diets of the control group (ESOIL0) (g/kg as-fed basis, unless otherwise indicated)

Ingredients	Starter Days 1–11	Grower Days 12–38	Finisher Days 39–45
Maize	486.2	513.4	562.1
Wheat, soft	100.0	100.0	100.0
Soybean meal (470 g/kg crude protein)	330.5	297.1	241.0
Flaxseed (230 g/kg crude protein)	10.0	10.0	10.0
Soybean oil	26.2	37.7	45.0
Dicalcium phosphate	15.2	13.0	12.2
Limestone (calcium carbonate)	15.1	13.9	12.5
Sodium chloride	3.2	3.2	3.2
Sodium carbonate	0.7	0.8	0.8
Lysine	4.2	3.3	5.9
Methionine	4.3	3.7	3.5
Threonine	1.9	1.4	1.3
Vitamin and mineral premix[1]	2.5	2.5	2.5
Nutrient content			
AME[2] (MJ/kg)	12.56	12.98	13.40
Crude protein	220.0	205.0	185.0
Crude fat	51.6	63.3	71.3
Crude fibre	26.2	25.5	24.0
Moisture	116.1	115.8	115.7
Ash	61.4	56.6	51.7
Calcium	9.6	8.7	7.9
Total phosphorus	7.0	6.4	6.0

[1] Supplying per kg feed: 13,000 IU vitamin A, 4,000 IU vitamin D3, 40 mg vitamin E, 9 mg vitamin K, 3 mg thiamine, 7 mg riboflavin, 6 mg pyridoxine, 0.035 mg vitamin B12, 40 mg niacin, 13 mg pantothenic acid, 1.5 mg folic acid, 0.13 mg biotin, 340 mg choline chloride, 55 mg Zn, 155 mg Mn, 20 mg Fe, 12 mg Cu, 0.2 mg Co, 1 mg I, 0.2 mg Se, and phytase 500 FTU

[2] AME: apparent metabolizable energy

For the essential oil gas chromatography–mass spectrometry (GC–MS) analysis of the feed additives, samples from the finisher diets were processed as described in European Pharmacopoeia (Council of Europe, 2005). Samples from the three EO oils were analysed using a GC system (Shimadzu GC-2010-GCMS-QP2010, Japan) equipped with an HP INNOWAX capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm). The identification of the components was performed by comparison with literature data (Adams, 2007).

The antioxidant activity of the EOs in the feed additives and the diets was determined according to Peperidou *et al.* (2014) at 517 nm with an UV-visible spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan). The diets and herbal feed additives were analysed for their total phenolic content according to Kiritsakis *et al.* (2010). The absorbance against the reagent blank was determined at 750 nm.

The bacterial isolation, enumeration, and identification were performed according to established methods (Tzora *et al.*, 2021). MacConkey and Kanamycin aesculin azide (KAA) agar (Merck, Darmstadt, Germany) was used to isolate *Escherichia coli* and *Enterococci,* respectively (Sanlibaba *et al.*, 2018). De Man, Rogosa, and Sharpe (MRS) agar (Oxoid, Basingstoke, UK), M17 agar (Lab M, Ltd, Lancashire, UK) and Tryptose sulphite cycloserine (TSC) agar (Merck, Darmstadt, Germany) were used to isolate *Lactobacilli, Lactococci,* and *Clostridium perfringens*, respectively. *Bifidobacterium* isolation and enumeration were performed on trans-oligosaccharide propionate agar medium (TOS) (Merck, Darmstadt, Germany) supplemented with glacial acetic acid (1%, v/v) and

mupirocin (100 µl/ml). Plate count agar medium (Oxoid, Basingstoke, UK) was used for the total aerobic and anaerobic counts.

Identification of the bacterial isolates was executed using a Bruker MALDI biotyper (Bruker Daltonics) following standard methods (Bujnakova *et al.*, 2013; Dec *et al.*, 2016; Shell *et al.*, 2017). The mass spectra were handled by the MALDI Biotyper 3.0 software package (Bruker, Leipzig, Germany). Identification of isolate strains and dendrogram plots were done as described by the manufacturer (Pokorna *et al.*, 2019; Dec *et al.*, 2021).

Morphometric analysis of the small intestine was evaluated according to Gava *et al.* (2015). Images were evaluated under light microscopy, with a Nikon microscope coupled with a Microcomp integrated digital imaging analysis system (Nikon Eclipse 200, Tokyo, Japan) and Image-Pro Plus analysis software (Figure 1). Villus height (VH) and crypt depth (CD) were calculated as the mean of 10 values.

For the analysis of the meat chemical composition, initially carcasses were handled according to commercial practice. Then, breast and thigh meat samples of 200 g were deboned and ground with an industrial large meat grinder, and then analysed for moisture, crude protein and fat content using near infra-red spectroscopy on a FoodScan™ Lab (FOSS, Denmark), as described in AOAC 2007.04 (Anderson, 2007; AOAC, 2007).

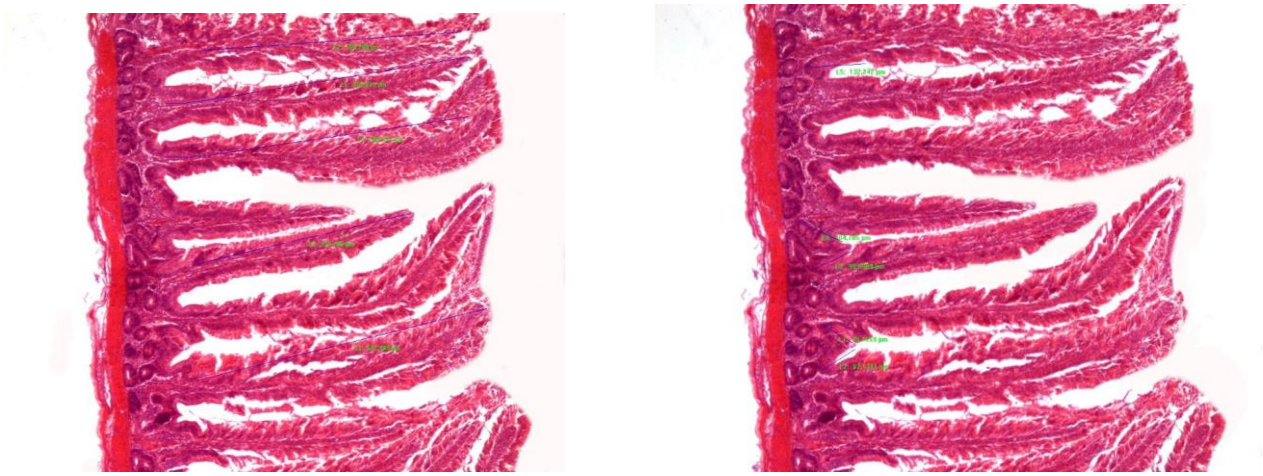
Lipid oxidation status of meat samples was determined according to Ahn *et al*. (1999) with minor modifications, with a spectrophotometer (UV 1700 PharmaSpec, Shimadzu, Japan) set at 532 nm. Lipid oxidation was determined as the 2-thiobarbituric acid-reactive substance (TBARS) value, expressed as mg malondialdehyde/kg of meat.

For the statistical analysis, the basic study design was a randomised complete blocks design. The replication (pen) was used as the experimental unit. Microbiology results were log-transformed (log_{10}) before the statistical analysis. Data homogeneity was examined using Levene's test. To evaluate the possible significant effects of the incremental EO supplementation (0, 100, 200, 400), the linear and quadratic effects ($P < 0.05$) were evaluated using the curve estimation regression process of the SPSS statistical package (Version 20, IBM SPSS). In addition, to identify specific supplementation that resulted in significant effects ($P < 0.05$), analysis of variance (ANOVA) and Tukey's post-hoc test were performed with the general linear model (univariate) process of the SPSS statistical package.

Results and Discussion

Table 2 and Figure 2 present the major components of the three EOs identified using GC-MS. In the oregano EO, 22 compounds were identified, the major being carvacrol, followed by thymol and p-cymene. In the sage EO, 47 compounds were identified, the major being eucalyptol, followed by β-pinene and α-pinene. Lastly, in the lavender EO, 55 compounds were identified, the major being linalool, followed by linalool acetate and cis-β-ocimene.

The results of the DPPH and Folin-Ciocalteu assay analyses are presented in Table 3. In the four feeds used in this trial, the total phenolic content varied between 18.02 mg/L in the control ESOIL0 feed extract and 66.25 mg/L in the ESOIL400 feed extract. The ESOIL400 extract showed the highest interaction with the stable radical DPPH, followed by the ESOIL200 extract. For samples ESOIL100, ESOIL200 and ESOIL400, the reducing activity was increased over time and found to be higher in all cases after 60 min of interaction, compared to 20 min. The extract of the control treatment, ESOIL0, showed the lowest concentration of phenol according to the Folin-Ciocalteu test results, with an average antioxidant activity. None of the samples had better antioxidant activity than trolox.



A B

Figure 1 Morphometric analysis of the epithelium of the jejunum of broiler chickens, showing villus height (A) and crypt depth (B) determination

No differences *(P* >0.05*)* were noted in live weight, feed intake, and FCR for the three periods (starter, grower, and finisher) or for the total trial (Table 4). Moreover, the EPEF productivity index was not affected (*P* >0.05).

The microbial populations of the intestinal digesta in the jejunum and the cecum were affected by feed supplementation (Table 5). In the jejunum, the EO supplementation resulted in a quadratic effect (*P* =0.011) on total aerobic bacteria (ESOIL200 was higher than the other treatments); linear decrease (P = 0.049) and quadratic effect (P = 0.019) on total anaerobic bacteria (ESOIL200 showed lower value compared with all other treatments); linear increase (P = 0.023) on *Enterobacteriaceae* (ESOIL100 and ESOIL400 were higher than the two other treatments); quadratic effect (*P* = 0.043) on *Lactobacillus* spp.; ESOIL200 was lower than ESOIL100 and ESOIL400. No linear or quadratic effects were found (*P* >0.050) for jejunum *Enterococcus* spp. In the cecum, the EO supplementation resulted in a linear increase (P <0.001) and quadratic effect (*P* <0.001) in total anaerobic bacteria (ESOIL400 was higher than the other three treatments); linear increase in *Enterococcus spp* (H). No linear or quadratic effects (*P* >0.05) were found for cecum total aerobic bacteria, *Enterobacteriaceae* and *Lactobacillus* spp*.* However, the ANOVA of the jejunum total aerobic bacteria showed that ESOIL200 had lower (*P* = 0.011) counts than ESOIL100 and ESOIL400.

Taxonomic characterization of bacterial species was performed using MALDI-TOF MS analysis. Of all 217 strains identified to species level (log(score) 1.7–3.0), 79 were identified as *E. coli*. Among the 81 *Lactobacilli* isolates, 15 (18.5%) were identified as *L. crispatus*, 8 (9.9%) as *L. reuteri*, 32 (39.5%) as *L. salivarius*, 23 (28.4%) *as L. johnsonii*, 2 (2.5%) as *L. gallinarum,* and 1 (1.2%) as *L. pontis*. In terms of *Enterococcus spp*, 5 (10.4%) isolates were identified as *E. faecalis*, 37 (77.1%) as *E. faecium,* and 6 (12.5%) *as E. cecorum*. In addition, four *Bifidobacteria* isolates were identified as B*. pseudolongum* and 5 as *B. gallinarum*.

A dendrogram of all 217 bacterial mass spectra (MSPs) created in relation to their mass signals and peak intensities is shown in Figure 3. At an arbitrary distance level of 1000 (maximum dissimilarity), the bacteria isolates can be classified by their MSP dendrogram into five main clusters. Cluster 1 comprised *Lb. johnsonii*, *Lb. reuteri*, *Lb. crispatus,* and *Lb. gallinarum*. Cluster 2 included *B. gallinarum* and *B. pseudolomum*. Cluster 3 included *L. salivarius* and *L. pontis*. Cluster 4 included *E. faecalis*, *E. faecium,* and *E. cecorum*. Finally, cluster 5 comprised *E. coli*. At minor distance levels, each main bacterial group was subdivided in smaller sub-groups. For example, at a distance level of 1000, cluster 5 (*E. coli* group) was clearly separate from the four other clusters. At a distance level of approximately 900, cluster 4 (*E. faecalis* group) was definitely distinct from clusters 3, 2, and 1, (*L. salivarius*, *B. gallinarum,* and *L. johnsonii* groups, respectively). At a distance level of approximately 150, there was great similarity of species between bacteria groups.

Table 2 Major components (%) of the essential oils of oregano, sage and lavender, identified by gas chromatography/mass spectrometry and listed in order of elution

	Compounds	OEO	SEO	LEO		Compounds	OEO	SEO	LEO		Compounds	OEO	SEO	LEO
1	α-Pinene	-	4.17	0.36	27	Heptyl acetate	-	-	0.02	53	β-Ocimene	0.11	-	-
2	α-Thujene	1.05	0.59	0.17	28	Octen-1-ol, acetate	-	0.04	0.90	54	α-Caryophyllene	-	0.55	0.04
3	Camphene	0.07	1.14	0.18	29	3-Octanol	-	-	0.38	55	Cryptone	-	-	0.17
4	Butyl acetate	-	-	0.08	30	*cis*-Thujone	-	3.46	-	56	Isothujol	-	0.06	-
5	β-Thujene	0.06	-	-	31	Butyl hexanoate	-	-	0.05	57	β-Farnesene	-	-	1.82
6	β-Pinene	-	11.44	0.05	32	n-Hexyl butanoate	-	-	0.51	58	Myrcenol	-	0.57	-
7	Sabinene	-	0.84	0.05	33	*trans*-thujone	-	3.66	-	59	Lavandulol	-	-	0.76
8	Butyl isobutyrate	-	-	0.01	34	Hexyl 2-methylbutyrate	-	-	0.03	60	α-Terpineol acetate	-	2.48	-
9	α-Phellandrene	0.08	-	0.05	35	*cis*-Linaloloxide (furanoid)	-	0.01	0.09	61	Borneol	0.69	-	-
10	β-Myrcene	0.71	5.31	1.72	36	α-Cubebene	-	0.02	-	62	γ-Muurolene	-	-	0.28
11	α-Terpinene	0.67	0.33	0.07	37	1-Octen-3-ol	0.45	-	0.21	63	α-Terpineol	-	2.04	1.81
12	D-Limonene	-	1.48	0.57	38	*trans-Sabinene hydrate*	0.17	0.25	-	64	*cis*-Sabinol	-	0.20	-
13	β-Phellandrene	0.12	-	-	39	*trans*-Linaloloxide (furanoid)	-	-	0.07	65	β-Bisabolene	1.10	-	-
14	Eucalyptol	-	47.89	1.34	40	Copaene	-	0.04	-	66	Nerol acetate	-	0.05	0.66
15	n-Butyl butanoate	-	-	0.26	41	Camphor	-	2.04	0.15	67	Germacrene D	-	0.11	0.01
16	*cis*-β-Ocimene	-	-	5.55	42	β-Bourbonene	-	0.05	-	68	Geraniol acetate	-	0.07	1.10
17	γ-Terpinene	3.65	0.79	0.17	43	Zingiberene	-	-	0.04	69	Nerol	-	-	0.17
18	*trans*-β-Ocimene	-	0.06	4.51	44	*cis*-Sabinenehydrate	-	0.10	-	70	Calamenene	-	0.03	-
19	3-Octanone	-	0.11	2.31	45	Linalool	0.12	1.23	35.55	71	Geraniol	-	0.04	0.42
20	p-Cymene	5.98	0.79	0.07	46	Linalool acetate	-	1.61	25.00	72	Caryophyllene oxide	-	0.12	-
21	Terpinolene	-	0.15	0.18	47	β-Caryophyllene	1.35	2.97	2.78	73	Epiglobulol	-	0.15	-
22	Hexyl acetate	-	-	1.36	48	Aromadendrene	-	0.13	-	74	Thymol	6.56	0.15	0.20
23	3-Octanol, acetate	-	-	0.29	49	1-Terpinen-4-ol	0.57	0.48	2.28	75	Hinesol	0.38	0.07	0.08
24	Hexyl isobutyrate	-	-	0.08	50	Thymol methyl ether	0.32	-	-	76	Carvacrol	75.06	0.92	1.31
25	1-Hexanol	-	-	0.11	51	Nerol acetate	-	-	2.62	77	Apiol	0.50	0.08	0.11
26	*allo*-Ocimene	-	-	0.10	52	β-Terpineol acetate	-	0.07	-					

OEO: oregano essential oil; SEO: sage essential oil; LEO: lavender essential oil

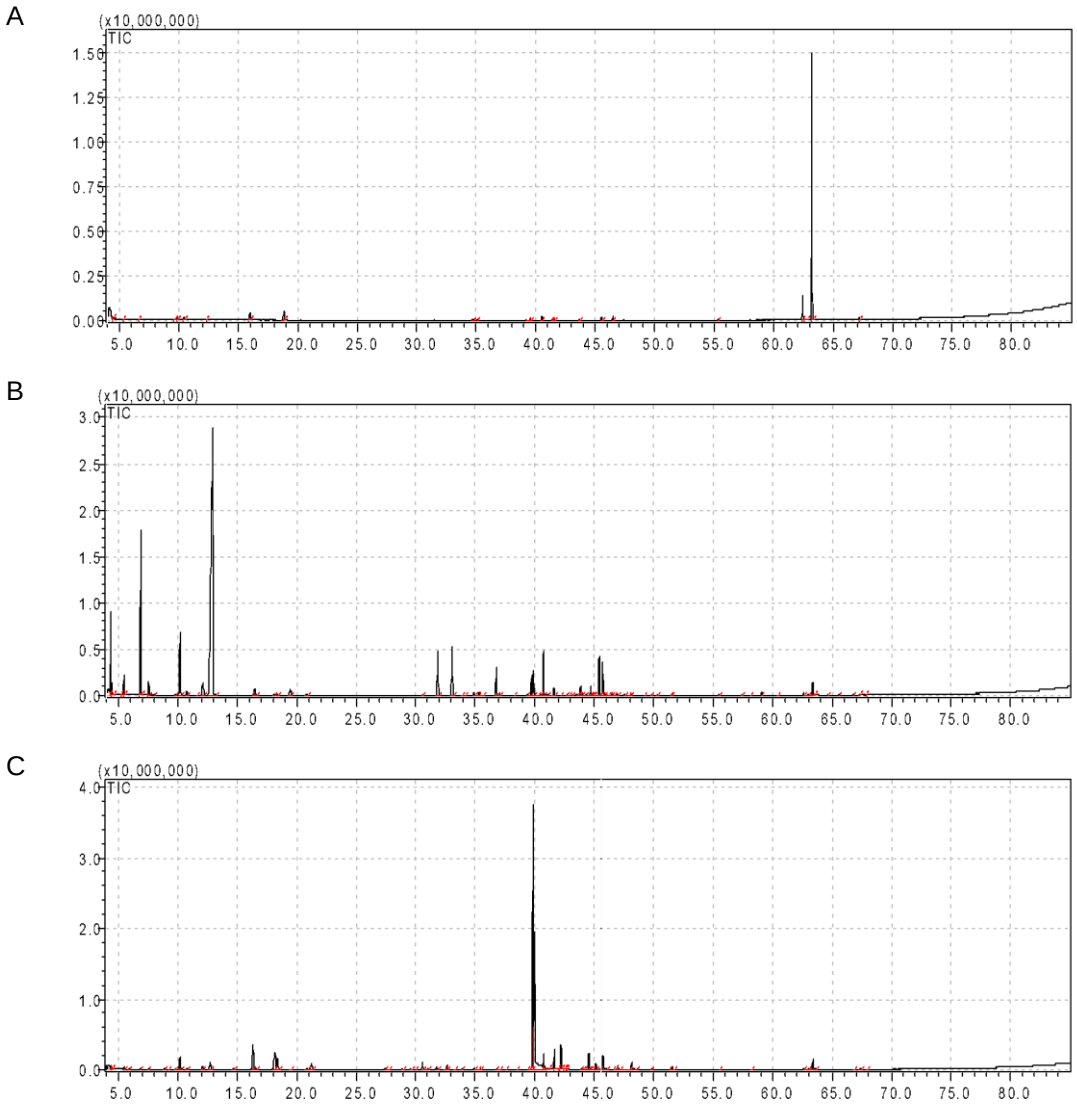


Figure 2 Gas chromatography–mass spectrometry analysis of the constituents (retention time x total ion chromatogram) of three essential oils: A) oregano; B) sage; C) lavender

Table 3 Antioxidant activity of dietary essential oils and diets

	DPPH (20 mg/ml)		FC/TF
	20 min	60 min	GA mg/l
Oregano essential oil	87.70	93.55	837.67
Sage essential oil	10.98	11.62	3.83
Lavender essential oil	22.15	25.87	1.02
ESOIL0	46.23	44.56	18.23
ESOIL100	56.45	61.95	43.31
ESOIL200	62.36	69.56	50.43
ESOIL400	75.27	85.87	66.24
Reference compound (nordihydroguaiaretic acid)	81	93	

DPPH: radical-scavenging ability determination, using stable radical 1.1-diphenyl-2-picrylhydrazyl; FC/TP: total phenolic content determination using Folin-Ciocalteu assay; GAE: gallic acid equivalents
ESOIL0: control non-supplemented treatment; ESOIL100: feed supplemented with 50 mg oregano EO + 25 mg sage EO + 25 mg lavender EO/kg; ESOIL200: feed supplemented with 100 mg oregano EO + 50 mg sage EO + 50 mg lavender EO/kg; ESOIL400: feed supplemented with 200 mg oregano EO + 100 mg sage EO + 100 mg lavender EO/kg

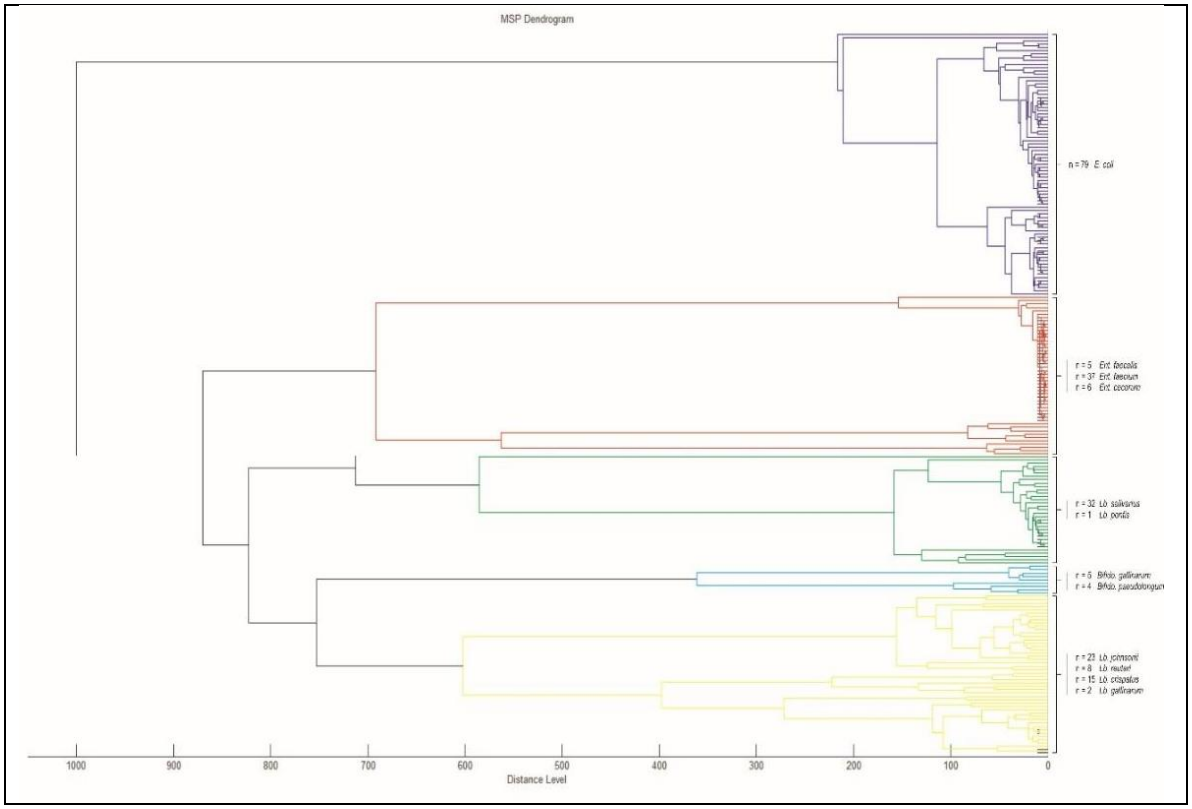


Figure 3 Dendrogram showing five clusters obtained by analysis of mass spectral profiles from bacterial species isolated from chickens.
In the bacterial mass spectra dendrogram, relative distance between isolates is displayed as arbitrary units. Zero indicates complete similarity and 1000 indicates maximum dissimilarity

Table 6 presents the results of the jejunum architecture analysis. No linear or quadratic effect (P >0.050) was noted for jejunum villus height, crypt depth, or villus height to crypt depth ratio. However, the ANOVA showed that ESOIL100 had a shorter (P =0.015) villus height than ESOIL400.

The results of the chemical analysis of chicken breast and thigh meat are shown in Table 7. In the breast meat, EO supplementation caused a quadratic effect (P <0.001) on the ash percentage (ESOIL200 showed higher values than all other treatments). Parameters such as moisture, protein, and fat percentages were not affected (P >0.05). In the thigh meat, EO supplementation caused a linear decrease (P =0.002) and quadratic effect (P =0.007) on protein percentage. ESOIL400 showed lower values compared with ESOIL0 and ESOIL100. Other thigh meat parameters such as moisture, fat, and ash percentage were not affected (P >0.05).

Determination of TBARS in breast and thigh meat kept under refrigerated storage (4 °C) is shown in Table 7. Regarding the breast meat, TBARS values showed a linear decrease (P<0.001) and a quadratic effect (P <0.001) after one day of storage (ESOIL200 and ESOIL400 had lower values compared with the other two treatments, and ESOIL100 also had lower value compared with ESOIL0); moreover, breast meat TBARS values showed a linear decrease (P = 0.001) and a quadratic effect (P <0.001) after 4 days of storage (ESOIL100, ESOIL200, and ESOIL400 had lower values compared to the control, ESOIL0). Regarding the thigh meat, TBARS values showed a linear decrease (P <0.001) and a quadratic effect (P<0.001) after 1 day of storage (ESOIL100, ESOIL200, and ESOIL400 had lower values than the control, ESOIL0); similarly, TBARS values showed a linear decrease (P = 0.002) and a quadratic effect (P <0.001) after 4 days of storage (ESOIL100, ESOIL200, and ESOIL400 had lower values compared to the control, ESOIL0).

Table 4 Effect of dietary supplementation with herbal essential oils on broiler chicken performance parameters (means & SEM)

Live body weight on day (g)	**ESOIL0**	**ESOIL100**	**ESOIL200**	**ESOIL400**	**SEM**	**ANOVA P**	**Linear regression P**	**Quadratic regression P**
1	38.8	38.1	38.3	38.7	0.11	0.150	0.968	0.132
14	368.6	376.1	368.6	373.9	4.00	0.878	0.788	0.965
28	1134.4	1157.5	1169.4	1183.6	9.27	0.314	0.065	0.163
42	2125.8	2138.9	2168.2	2203.5	26.83	0.746	0.259	0.537
Feed intake per chicken during period (g)								
1-14 days	345.0	344.2	330.3	346.1	10.60	0.945	0.993	0.887
15-28 days	1493.9	1529.7	1518.3	1496.7	22.63	0.931	0.911	0.836
29-42 days	2125.8	2138.9	2139.7	2203.5	29.24	0.785	0.320	0.587
1-42 days	3808.3	3981.9	3968.0	3782.5	43.64	0.264	0.587	0.144
Feed conversion ratio during period (g feed/ g weight gain)								
1-14 days	1.048	1.016	1.006	1.037	0.035	0.972	0.959	0.889
15-28 days	1.952	2.017	1.900	1.889	0.032	0.482	0.282	0.558
29-42 days	2.153	2.191	2.180	2.166	0.027	0.964	0.955	0.902
1-42 days	1.829	1.876	1.847	1.733	0.021	0.112	0.060	0.051
EPEF								
1-42 days	272.6	255.7	272.7	289.2	5.51	0.235	0.141	0.193

No of replicates: each treatment had 6 pens of 12 male birds/pen
ESOIL0: control non-supplemented treatment; ESOIL100: feed supplemented with 50 mg oregano EO + 25 mg sage EO + 25 mg lavender EO/kg; ESOIL200: feed supplemented with 100 mg oregano EO + 50 mg sage EO + 50 mg lavender EO/kg; ESOIL400: feed supplemented with 200 mg oregano EO + 100 mg sage EO + 100 mg lavender EO/kg.
EPEF: European production efficiency factor; SEM: standard error of mean

Table 5 Effect of dietary supplementation with herbal essential oils on broiler chickens' jejunum and cecum microflora (means & SEM)

Jejunum (Log CFU/g)	ESOIL0	ESOIL100	ESOIL200	ESOIL400	SEM	ANOVA P	Linear regression P	Quadratic regression P
Total aerobic bacteria	6.606 [a]	6.541 [a]	8.416 [b]	6.377 [a]	0.102	<0.001	0.826	0.011
Total anaerobic bacteria	8.425 [c]	8.744 [c]	5.752 [a]	7.165 [b]	0.136	<0.001	0.049	0.019
Enterobacteriaceae	4.166 [a]	6.511 [b]	4.880 [a]	6.389 [b]	0.130	<0.001	0.023	0.068
Enterococcus spp	6.379	6.176	5.256	5.692	0.140	0.064	0.099	0.067
Lactobacillus spp	7.068 [ab]	7.517 [b]	5.926 [a]	7.946 [b]	0.139	0.001	0.235	0.043
Cecum (Log CFU/g)								
Total aerobic bacteria	7.781 [ab]	6.791[a]	8.693 [b]	8.199 [b]	0.167	0.011	0.164	0.383
Total anaerobic bacteria	8.274 [a]	8.266 [a]	8.089 [a]	10.312 [b]	0.133	<0.001	<0.001	<0.001
Enterobacteriaceae	7.714	7.033	7.453	7.783	0.147	0.303	0.461	0.318
Enterococcus spp	6.589	6.594	7.087	7.046	0.075	0.054	0.025	0.061
Lactobacillus spp	7.386	7.589	7.382	7.492	0.091	0.837	0.862	0.980

No of replicates: each treatment had 6 pens of 12 male birds/pen.
ESOIL0: control non-supplemented treatment; ESOIL100: feed supplemented with 50 mg oregano EO + 25 mg sage EO + 25 mg lavender EO/kg; ESOIL200: feed supplemented with 100 mg oregano EO + 50 mg sage EO + 50 mg lavender EO/kg; ESOIL400: feed supplemented with 200 mg oregano EO + 100 mg sage EO + 100 mg lavender EO/kg; SEM: standard error of means; [a,b,c] Values in the same row without superscripts in common differ significantly (P <0.05)

Table 6 Effect of dietary supplementation with herbal essential oils on broiler chicken jejunum architecture (means & SEM)

Jejunum	ESOIL0	ESOIL100	ESOIL200	ESOIL400	SEM	ANOVA P	Linear regression P	Quadratic regression P
Villus height (nm)	719.9 [ab]	808.7 [b]	741.5 [ab]	689.0 [a]	9.87	0.015	0.169	0.063
Crypt depth (nm)	184.9	199.3	196.0	166.3	10.64	0.701	0.420	0.475
Villus height / Crypt depth	4.02	4.24	3.80	4.16	0.206	0.885	0.934	0.951

No of replicates: each treatment had 6 pens of 12 male birds/pen
ESOIL0: control non-supplemented treatment; ESOIL100: feed supplemented with 50 mg oregano EO + 25 mg sage EO + 25 mg lavender EO/kg; ESOIL200: feed supplemented with 100 mg oregano EO + 50 mg sage EO + 50 mg lavender EO/kg; ESOIL400: feed supplemented with 200 mg oregano EO + 100 mg sage EO + 100 mg lavender EO/kg.
SEM: standard error of means; [a,b] Values in the same row without superscripts in common differ significantly (P <0.05)

Table 7 Effect of dietary supplementation with herbal essential oils on broiler chicken breast and thigh meat composition and oxidative stability under refrigerated storage (4 °C) (means & SEM)

Breast meat composition (%)	**ESOIL0**	**ESOIL100**	**ESOIL200**	**ESOIL400**	**SEM**	**ANOVA P**	**Linear regression P**	**Quadratic regression P**
Moisture	74.55	74.79	74.85	74.81	0.084	0.654	0.406	0.439
Protein	23.65	23.38	23.12	23.37	0.105	0.421	0.459	0.244
Fat	1.81	1.51	1.14	1.57	0.105	0.230	0.592	0.132
Ash	0.62 [a]	0.85 [b]	1.06 [c]	0.84 [b]	0.024	0.001	0.216	<0.001
Thigh meat composition (%)								
Moisture	73.45	74.47	74.38	74.13	0.201	0.359	0.548	0.262
Protein	19.88 [b]	19.84 [b]	19.65 [ab]	19.05 [a]	0.089	0.023	0.002	0.007
Fat	6.24	5.26	5.45	6.06	0.167	0.181	0.727	0.126
Ash	1.09	1.08	1.07	1.21	0.039	0.526	0.203	0.318
Breast meat TBARS (ng/g)								
Day 1	4.790 [c]	3.655 [b]	2.431 [a]	2.683 [a]	0.111	<0.001	<0.001	<0.001
Day 4	6.515 [b]	4.226 [a]	3.754 [a]	3.730 [a]	0.171	<0.001	0.001	<0.001
Thigh meat TBARS (ng/g)								
Day 1	6.877 [b]	4.088 [a]	3.334 [a]	2.602 [a]	0.190	<0.001	<0.001	<0.001
Day 4	8.143 [b]	5.201 [a]	4.245 [a]	4.626 [a]	0.247	<0.001	0.002	<0.001

No of replicates: each treatment had 6 pens of 12 male birds/pen
ESOIL0: Control non-supplemented treatment; ESOIL100: feed supplemented with 50 mg oregano EO + 25 mg sage EO + 25 mg lavender EO/kg; ESOIL200: feed supplemented with 100 mg oregano EO + 50 mg sage EO + 50 mg lavender EO/kg; ESOIL400: feed supplemented with 200 mg oregano EO + 100 mg sage EO + 100 mg lavender EO/kg.
SEM: standard error of the means; TBARS: thiobarbituric acid reactive substances
[a,b,c] Values in the same row without superscripts in common differ significantly (P <0.05)

The health and productivity of broiler chickens are related to their intestinal microbiome, which may be influenced by parameters such as the age of the bird, the health condition of the gastrointestinal tract, and the use of feed components (Oakley *et al.*, 2014; Petricevic *et al.*, 2018). Thus, the microbiota of the avian gastrointestinal tract, which consists mainly of bacteria and protozoa, is critical to digestion, nutrient absorption, and immune response to pathogens. The balance of the enteric microbiota is a dynamic phenomenon depending on various parameters (Oakley *et al.*, 2014; Tzora *et al.*, 2021). Enteric microbiota population shifts may take place after an infection, with increases or decreases of some species of microorganisms (Oakley *et al.*, 2014; McDonald *et al.*, 2017; Tzora *et al.*, 2017). The results of the intestinal microflora composition, analysed with MALDI-TOF MS in the current study, agree in part with observations in which dietary EOs exhibited the ability to affect the taxa of beneficial *Lactobacilli* and *Bifidobacteria* positively (Liu *et al.*, 2017; Tzora *et al.*, 2021) and simultaneously had an inhibitory effect against opportunistic, harmful bacteria such as Coliforms, Enterobacteriaceae, *Campylobacter*, *Clostridium,* and *Bacteroides* spp. (Tzora *et al.*, 2017). Dendrogram analysis of the bacterial composition in the jejunum and cecum confirmed that the antimicrobial effect of EOs seemed to be selective for some categories of bacteria, as notable differences were found for total anaerobic bacteria, Enterobacteriaceae and *Lactobacilli*, supporting the importance of a quantitative analysis being accompanied by a qualitative analysis.

Numerous manuscripts have been published on dietary EOs and their effects on poultry performance, but with variable and inconsistent findings (Bozkurt *et al.*, 2013; Petricevic *et al.*, 2018; Abbasi *et al.*, 2020; Sevim *et al.*, 2020). This variability in EO efficacy can be attributed partially to the overall husbandry management and health status of the poultry, since the benefits are more apparent when the overall management and housing conditions are not optimal (Bonos *et al.*, 2010; Mesa *et al.*, 2017). Besides, many factors may affect this efficacy, such as EO composition, active ingredients, and synergistic interactions. The hydroxyl group of phenols confers antibacterial activity and its relative position in the molecule is critical to its effectiveness. This may be an explanation for the greater antibacterial activity of carvacrol, compared to the phenolics of other plants (Amer *et al.*, 2018). In the current study, the results of the essential oil phenolic content analyses agreed with those of the GC-MS analysis. The oregano EO was rich in carvacrol (75.06%) and thymol (6.56%), which are the main monoterpenoid phenols. Comparatively, the EOs of sage and lavender had only limited amounts of carvacrol (0,92% and 0.15%, respectively) and thymol (1.31% and 0.20%, respectively). The primary effect of EOs as a reducer of free radicals has been demonstrated extensively (Christaki *et al.*, 2012; Bozkurt *et al.*, 2013; Giannenas *et al.*, 2018; Cimrin *et al.*, 2020).

The health status of the intestine is important to ensure optimal nutrient utilization (Sugiharto *et al.*, 2021). Increased villus length and overall surface area are considered positive indicators of efficient digestive function, correlating with improved nutrient absorption (Sen *et al.*, 2011; Barbarestani *et al.*, 2020). Decreased crypt depth is linked to lower tissue turnover and reduced secretion (Sen *et al.*, 2011). The rate of replacement of the intestinal epithelium indicates a dynamic relationship between the multiplication of enterocytes in the crypts and their desquamation from the villus, which poses serious energy costs. Studies have shown that EO supplementation may affect duodenum, jejunum, and ileum intestinal morphology (Tzora *et al.*, 2017; Barbarestani *et al.*, 2020). The potential beneficial effect of dietary sage or lavender on intestinal microarchitecture and health has not been studied extensively in poultry. However, some recent results are promising (Barbarestani *et al.*, 2020; Farhadi *et al.*, 2020; Adaszynska-Skwirzynska *et al.*, 2021).

In the current study, a significant antioxidant effect was noticed in breast and thigh tissue in the EO-supplemented diets. Phytogenic products may influence the activity of antioxidant enzymes, which can decrease inflammatory agents, such as reactive oxygen species (ROS), protecting cells and tissues from oxidation, atrophy, and penetration of the membrane barrier (Moretti *et al.*, 2018). This might indicate lower requirements for other antioxidant defence mechanisms against lipid peroxidation. Dietary addition of Labiate family plants and their EOs can improve the storage quality of refrigerated poultry meat by protecting it from lipid oxidation (Surai, 2014; Barbarestani *et al.*, 2020). In the present trial, the authors found that the addition of mixed EOs in incremental levels increased dietary total phenolic content and decreased the TBARS values in the breast and thigh meat samples.

Conclusions

The present study showed that supplementation of broiler diets with a mixture of EOs from oregano, sage, and lavender at incremental levels modified the intestinal microflora and improved meat oxidative stability without affecting broiler performance parameters. In addition, the increased levels of supplementation enhanced the radical scavenging capacity of the supplemented feeds. It is worth investigating whether EOs could confer antimicrobial efficiency on a larger scale to define the

mechanisms of action in controlling diseases in farm animals, especially those caused by oxidation or microbial growth.

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Authors' Contributions
EB, IS conceived the idea; EB, IS, AT conducted the trial and collected the samples; IG, KF, ES, OT, DL, DG, IS, AT performed analysis; EB, IS, IG, DL, DG, AT analysed the data and wrote the article.

Conflict of Interest
The authors declare there is no conflict of interest.

References
Abbasi, M.A., Ghazanfari, S., Sharifi, S.D. & Gavlighi, H.A., 2020. Influence of dietary plant fats and antioxidant supplementation on performance, apparent metabolizable energy, and protein digestibility, lipid oxidation, and fatty acid composition of meat in broiler chicken. Vet. Med. Sci. 6, 54-68. DOI: 10.1002/vms3.212

Adams, R., 2007. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured, Carol Stream, IL.

Adaszynska-Skwirzynska, M., Szczerbinska, D. & Zych, S., 2021. The use of lavender *(Lavandula angustifolia)* essential oil as an additive to drinking water for broiler chickens and its *in vitro* reaction with enrofloxacin. Animals 11, 1535. DOI: 10.3390/ani11061535

Ahn, D.U., Olson, D.G., Jo, C., Love, J. & Jin, S.K., 1999. Volatile production and lipid oxidation on irradiated cooked sausage as related to packaging and storage. J. Food Sci. 64, 226-229. DOI: 10.1111/j.1365-2621.1999.tb15870.x

Amer, S.A., Metwally, A.E. & Ahmed, S.A.A., 2018. The influence of dietary supplementation of cinnamaldehyde and thymol on the growth performance, immunity, and antioxidant status of monosex Nile tilapia fingerlings *(Oreochromis niloticus).* Egyptian J. Aquatic Res. 44, 251-256. DOI: 10.1016/j.ejar.2018.07.004

Anderson, S., 2007. Determination of fat, moisture, and protein in meat and meat products by using the FOSS FoodScan near-infrared spectrophotometer with FOSS artificial neural network calibration model and associated database: Collaborative study. J. AOAC Int. 90, 1073-1083. DOI: 10.1093/jaoac/90.4.1073

AOAC, 2007. Official Methods of Analysis. 18th edition. Association of Analytical Chemists, AOAC International, Arlington, Virginia, USA.

Aviagen, 2019. ROSS Nutrition Specifications. Aviagen, Huntsville, USA.

Barbarestani, S.Y., Jazi, V., Mohebodini, H., Ashayerizadeh, A., Shabani, A. & Toghyani, M., 2020. Effects of dietary lavender essential oil on growth performance, intestinal function, and antioxidant status of broiler chickens. Livestock Sci. 233, 103958. DOI: 10.1016/j.livsci.2020.103958

Bonos, E., Christaki, E. & Florou-Paneri, P., 2010. Performance and carcass characteristics of Japanese quail as affected by sex or mannan oligosaccharides and calcium propionate. S. Afr. J. Anim. Sci. 40, 173-184. DOI: 10.4314/sajas.v40i3.2

Bozkurt, M., Giannenas, I., Kucukyilmaz, K., Christaki, E. & Florou-Paneri, P., 2013. An update on approaches to controlling coccidia in poultry using botanical extracts. Br. Poult. Sci. 54, 713-727. DOI: dx.doi.org/10.1080/00071668.2013.849795

Bujnakova, D., Strakova, E. & Kmet, V., 2013. *In vitro* evaluation of the safety and probiotic properties of *Lactobacilli* isolated from chicken and calves. Anaerobe 29, 118-127. DOI: 10.1016/j.anaerobe.2013.10.009

Christaki, E., Bonos, E., Giannenas, I. & Florou-Paneri, P., 2012. Aromatic plants as a source of bioactive compounds. Agriculture 2, 228-243. DOI: 10.3390/agriculture2030228

Cimrin, T., Ivgin Tunca, R., Avsaroglu, M.D., Ayasan, T. & Kucukersan, S., 2020. Effects of an antibiotic and two phytogenic substances (cinnamaldehyde and 1,8-cineole) on yolk fatty acid profile and storage period-associated egg lipid peroxidation level. Rev. Bras. Zootec. 49, e20190270. DOI: 10.37496/rbz4920190270

Council of Europe, 2005. In: European Pharmacopoeia, 5th edition. COE, Strasbourg. Pp. 2710-2711.

Dec, M., Puchalski, A., Urban-Chmiel, R. & Wernicki, A., 2016. 16S-ARDRA and MALDI-TOF mass spectrometry as tools for identification of *Lactobacillus* bacteria isolated from poultry. BMC Microbiol. 16, 105. DOI: 10.1186/s12866-016-0732-5

Dec, M., Stepien-Pysniak, D., Puchalski, A., Hauschild, T., Pietras-Ozga, D., Ignaciuk, S. & Urban-Chmiel, R., 2021. Biodiversity of *Ligilactobacillus salivarius* strains from poultry and domestic pigeons. Animals (Basel) 11, 972. DOI: 10.3390/ani11040972

Farhadi, M., Hedayati, M., Manafi, M. & Khalaji, S., 2020. Influence of using sage powder *(Salvia officinalis)* on performance, blood cells, immunity titers, biochemical parameters, and small intestine morphology in broiler chickens. Iranian J. Appl. Animal. Sci. 10, 509-516.

Franciosini, M.P., Casagrande-Proietti, P., Forte, C., Beghelli, D., Acuti, G., Zanichelli, D., dal Bosco, A., Castellini, C. & Trabalza-Marinucci, M., 2016. Effects of oregano (*Origanum vulgare L*.) and rosemary (*Rosmarinus officinalis L*.) aqueous extracts on broiler performance, immune function and intestinal microbial population. J. Appl. Animal. Res. 44, 474-479. DOI: 10.1080/09712119.2015.1091322

Gava, M.S., Moraes, L.B., Carvalho, D., Chitolina, G.Z., Fallavena, L.C.B., Moraes, H.L.S., Herpich, J. & Salle, C.T.P., 2015. Determining the best sectioning methods and intestinal segments for morphometric analysis in broilers. Braz. J. Poult. Sci. 71, 145-150. DOI: 10.1590/1516-635x1702145-150

Giannenas, I., Bonos, E., Skoufos, I., Tzora, A., Stylianaki, I., Lazari, D., Tsinas, A., Christaki, E. & Florou-Paneri, P., 2018. Effect of herbal feed additives on performance parameters, intestinal microbiota, intestinal morphology, and meat lipid oxidation of broiler chicken. Br. Poult. Sci. 59, 545-553. DOI: 10.1080/00071668.2018.1483577

Huyghebaert, G., Ducatelle, R. & Van Immerseel, F., 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187, 182-188. DOI: 10.1016/j.tvjl.2010.03.003

Kiritsakis, K., Kontominas, M.G., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A. & Kiritsakis, A., 2010. Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. J. Am. Oil Chem. Soc. 87, 369-376. DOI: 10.1007/s11746-009-1517-x

KPI Library, 2021. https://kpilibrary.com/kpis/european-production-efficiency-factor-epef-2.

Liu, Y., Yang, X., Xin, H., Chen, S., Yang, C., Duan, Y. & Yang, X., 2017. Effects of a protected inclusion of organic acids and essential oils as antibiotic growth promoter alternative on growth performance, intestinal morphology, and gut microflora in broilers. Animal Sci. J. 88, 1414-1424. DOI: 10.1111/asj.12782

McDonald, S.E., Nolan, M.J., Harman, K., Boulton, K., Hume, D.A., Tomley, F.M., Stabler, R.A. & Blake, D.P., 2017. Effects of *Eimeria tenella* infection on chicken caecal microbiome diversity, exploring variation associated with severity of pathology. PLOS One 12, e0184890. DOI: 10.1371/journal.pone.0184890

Mesa, D., Muniz, E., Souza, A. & Geffroy, B., 2017. Broiler-housing conditions affect the performance. Rev. Bras. Cien. Avic. 19, 263-272. DOI: 10.1590/1806-9061-2016-0346

Moretti, S., Mrakic-Sposta, S., Roncoroni, L., Vezzoli, A., Dellanoce, C., Monguzzi, E., Branchi, F., Ferretti, F., Lombardo, V., Duoneda, L., Scricciolo, A. & Luca, E., 2018. Oxidative stress as a biomarker for monitoring treated celiac disease. Clin. Transl. Gastroenterol. 9, 157. DOI: 10.1038/s41424-018-0031-6

NRC, 1994. Nutrient Requirements of Poultry. 9th rev. National Academy Press, Washington, USA.

Oakley, B., Lillehoj, H.S., Kogut, M.H., Kim, W.K., Maurer, J.J., Pedroso, A.A., Lee, M.D., Collett, S.R., Johnson, T.J. & Cox, N.A., 2014. The chicken gastrointestinal microbiome. FEMS Microbiol. Lett. 360, 100-112. DOI:

Peperidou, A., Kapoukranidou, D., Kontogiorgis, C. & Hadjipavlou-Litina, D., 2014. Multitarget molecular hybrids of cinnamic acids. Molecules 19, 20197-20226. DOI: 10.3390/molecules191220197

Petracci, M., Bianchi, M., Mudalal, S. & Cavani, C., 2013. Functional ingredients for poultry meat products. Trends Food Sci. Technol. 33, 27-39. DOI: 10.1016/j.tifs.2013.06.004

Petricevic, V., Lukic, M., Skrbic, Z., Rakonjac, S., Doskovic, V., Petricevic, M. & Stankojkovic, A., 2018. The effect of using rosemary *(Rosmarinus officinalis)* in broiler nutrition on production parameters, slaughter characteristics, and gut microbiological population. Turkish J. Vet. Anim. Sci. 42, 658-664. DOI: 10.3906/VET-1803-53

Pokorna, A., Manakova, T. & Cizek, A., 2019. Properties of potentially probiotic *Lactobacillus* isolates from poultry intestines. Acta Vet. Brno 88, 73-84. DOI: 10.2754/avb201988010073

Premier Nutrition, 2014. Premier Atlas 2014. Ingredients matrix. Premier Nutrition, Brereton, UK.

Presidential Decree, 2013. Presidential decree 56/2013 on harmonization of the directive 2010/63/EU, on the protection of animals used for scientific purposes. Greek Government, Athens, Greece.

Sanlibaba, P., Tezel, B.U. & Senturk, E., 2018. Antimicrobial resistance of *Enterococcus* species isolated from chicken in Turkey. Korean J. Food Sci. Anim. Resour. 38, 391-402. DOI: 10.5851/kosfa.2018.38.2.391

Sen, S., Ingale, S.L., Kim, Y.W., Kim, J.S., Lohakare, J.D., Kim, E.K., Kim, H.S., Ryu, M.H., Kwon, I.K. & Chae, B.J., 2011. Effect of supplementation of *Bacillus subtilis* LS 1–2 to broiler diet on growth performance, nutrient retention, caecal microbiology, and small intestinal morphology. Res. Vet. Sci. 93, 264-268. DOI: 10.1016/j.rvsc.2011.05.021

Sevim, B., Gumus, R., Harman, H., Ayasan, T., Baser, E., Altay, Y. & Akbulut, K., 2020. Effects of dietary rosemary essential oil on growth performance, carcass traits, and some hematological values of chukar partridge. Turkish J. Agric.-Food Sci. Technol. 8, 430-435. DOI: 10.24925/turjaf.v8i2.430-435.3121

Shell, W.S., Sayed, M.L., Allah, F.M.G., Gamal, F.E.M., Khedr, A.A., Samy, A.A. & Ali, A.H.M., 2017. Matrix-assisted laser desorption-ionization-time-of-flight mass spectrometry as a reliable proteomic method for characterization of *Escherichia coli* and *Salmonella* isolates. Vet. World 10, 1083. DOI: 10.14202/vetworld.2017.1083-1093

Sugiharto, S., Pratama, A.R., Yudiarti, T. & Ayasan, T., 2021. Effect of novel natural feed additive containing *Averrhoa bilimbi* L. fruit filtrate, wheat bran, and *Saccharomyces cerevisiae* on growth performance and meat characteristics of broilers. Vet. World 14, 3007-3014. DOI: 10.14202/vetworld.2021.3007-3014

Surai, P.F., 2014. Polyphenol compounds in the chicken/animal diet: from the past to the future. J. Anim. Physiol. Animal Nutr. 98, 19-31. DOI: 10.1111/jpn.12070

Tzora, A., Giannenas, I., Karamoutsios, A., Papaioannou, N., Papanastasiou, D., Bonos, E., Skoufos, S., Bartzanas, T. & Skoufos, I., 2017. Effects of oregano, attapulgite, benzoic acid and their blend on chicken performance, intestinal microbiology and intestinal morphology. J. Poultry Sci. 54, 218-227. DOI: 10.2141/jpsa.0160071

Tzora, A., Bonos, E., Giannenas, I., Karamoutsios, A., Fotou, K., Sidiropoulou, E., Tsiftsoglou, O.S., Lazari, D., Stylianaki, I., Grigoriadou, K. & Skoufos, I., 2021. Effects of dietary essential oils in combination with butyrate on growth performance, intestinal microflora, blood parameters, and meat composition of broiler chickens. Eur. Poultry Sci. 85, 1-24. DOI: 10.1399/eps.2021.327

Ullah, F., Ayaz, M., Sadiq, A., Ullah, F., Hussain, I., Shahid, M., Yessimbekov, Z., Adhikari-Devkota, A. & Devkota, H.P., 2020. Potential role of plant extracts and phytochemicals against foodborne pathogens. Appl. Sci. 10, 4597. DOI: 10.3390/app10134597

Valenzuela-Grijalva, N.V., Pinelli-Saavedra, A., Muhlia-Almazan, A., Dominguez-Diaz, D. & Gonzalez-Rios, H., 2017. Dietary inclusion effects of phytochemicals as growth promoters in animal production. J. Animal Sci. Technol. 59, 8. DOI: 10.1186/s40781-017-0133-9

Voidarou, C., Vassos, D., Kegos, T., Koutsotoli, A., Tsiotsias, A., Skoufos, J., Tzora, A., Maipa, V., Alexopoulos, A. & Bezirtzoglou, E., 2007. Aerobic and anaerobic microbiology of the immersion chilling in poultry. Poult. Sci. 86, 1218-1222. DOI: 10.1093/ps/86.6.1218

Wilson, D.W., Nash, P., Buttar, H.S., Griffiths, K., Singh, R., De Meester, F., Horiuchi, R. & Takahashi, T., 2017. The role of food antioxidants, benefits of functional foods, and influence of feeding habits on the health of the order person: An overview. Antioxidants 6, 81. DOI: 10.3390/antiox6040081

Zeng, Z., Zhang, S., Wang, H. & Piao, X., 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: A review. J. Anim. Sci. Biotechnol. 6, 1-10. DOI: 10.1186/s40104-015-0004-5