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Effect of Basil, Thyme and Sage Essential Oils as Phytogenic Feed Additives on Production Performances, Meat Quality and Intestinal Microbiota in Broiler Chickens

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Abstract: As the use of antibiotics has been banned or reduced in certain countries in animal industries, the search for new alternatives to antibiotics has been and will continue to be a research subject in poultry for several years. This study aimed to evaluate the effect of basil, thyme and sage essential oils (EO) in broiler chickens' diets. A total of 120 Cobb 500 broiler chickens aged 12 days were distributed into four homogeneous groups of thirty chickens with six replicates of five broilers each, raised until 42 days of age. For the diets, corn, soybean meal, corn gluten and wheat were used as the main ingredients for the control diet (CON), and three experimental diets were formulated as follows: a diet containing 0.05% essential oil from basil (EOB), a diet containing 0.05% essential oil from thyme (EOT), and a diet containing 0.05% essential oil from sage (EOS). The results showed that production performances, European Production Efficiency Factor (EPEF), European Broiler Index (EBI) and carcass weight were improved ($p < 0.05$) in the experimental groups compared with the control; however, no significant effect in anatomical parts development was observed. Lightness (L *) and hue angle (H *) colorimetric meat parameters were ($p < 0.05$) altered but without an effect on meat texture. The antioxidant capacity and total polyphenols content in the thigh meat and total n-3 and n-6 polyunsaturated fatty acids were higher in the experimental samples compared with the control. The intestinal microbiota was also significantly altered with a lowering of *Escherichia coli*, *Coliforms* and staphylococci in the small intestine and caecum and an increased lactobacilli count in the experimental groups compared with the control. Overall, all EO-supplemented diets showed the potential to improve meat quality; however, EOS was more effective in altering the chicken microbiota in the small intestine and caecum.

Keywords: basil; broiler chicken; essential oil; feed additive; sage; thyme; meat quality; intestinal microbiota

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1. Introduction

Because of growing concerns about food safety, environmental contamination and general health risks, more and more antibiotics have been banned as growth promoters in animal feed in recent years. As a result, animal scientists and producers have been looking for alternatives that promote growth, improve meat quality and boost animals' health. Plant extracts are currently receiving a lot of interest as feed additives due to their benefit of being natural. In the current world, knowing how to effectively utilize wild plants, particularly their extracts, is crucial. In the last two decades, innovative nutritional strategies have been explored for a variety of sources, including phytogenic feed additives

(PFA), that can be added to broiler chicken feed to benefit both the animal and the final product. Literature data revealed that, among studied PFA that include pre/probiotics [1,2], plants [3–5], vegetable wastes [6–8] and synthetic supplements, essential oils (EO) have a special place.

The EO are complex mixtures of volatile compounds formed by dozens of components, such as terpenoids (menthol, linalool, geraniol, borneol, α-terpineol) and low molecular weight aliphatic hydrocarbons (thymol, carvacrol, eugenol, cinnamaldehyde), produced by living organisms and isolated (pressing and distillation) from a whole plant or specific parts of plants with a known taxonomic origin [9]. After the ban of antibiotics in animals' diets in the European Union in 2006, the pressure to replace them with safe feed additives led researchers to conduct a massive exploration into utilizing non-antibiotic substances with growth-promoting potential including EO, which have received more attention after numerous PFA have been recognized as safe (GRAS) by the Food and Drug Administration [5]. It was estimated that, out of 3000 known EO, over 300 present high commercial interest in different industries [10]. Their use in broiler diets is a new issue, and there is an enormous variety of these products. Among these, the EO extracted from *Lamiaceae* or *Labiatae*, such as basil *(Ocimum basilicum)*, thyme *(Thymus vulgaris)* and sage (*Salvia officinalis*), present a rising interest to be used as PFA in chickens' diets. Previous studies reported that the utilization of different EO alone or combined into broiler chicken feeds had a positive effect on production performances [11,12], immunological status [13,14], enzymatic activity in blood serum and blood lipid profile [15,16], and intestinal morphology [17,18]. However, little was emphasized about their effect on meat quality.

Given the importance and relevance of EO in the last two decades, it is scientifically justified and interesting to examine the effects of using selected natural PFA in broiler chicken diets on production performances, meat quality and the impact on animals' intestinal health. This study was conducted to assess the potential phytogenic effect of basil, thyme and sage essential oils as possible candidates to improve broiler chicken production performances and efficiency, meat quality and intestinal microbiota.

2. Materials and Methods

2.1. Ethics, Supplements, Birds, Diets and Experimental Design

The experimental procedures used in this trial were approved by the ethical committee of the institute following Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, Law 43/11.04.2014, Directive 2010/63/EU).

Basil (*Ocimum basilicum*), thyme (*Thymus vulgaris*) and sage (*Salvia officinalis*) essential oils were purchased from a local medicinal agency (Bucharest, Romania).

A total of 120 Cobb 500 broiler chickens aged 12 days were divided into 4 uniform groups, each with 30 chickens and 6 replicates of 5 broilers each. They were raised until the feeding experiment was completed at 42 days of age. For the diets, corn, soybean meal, corn gluten and wheat were used as the main ingredients for all control diets (CON) in each raising phase, as shown in Table 1, and were formulated to be isonitrogenous, isoenergetic and isofibrous to meet the nutritional requirements of Cobb 500 broiler chickens. Three experimental diets were formulated as follows: a diet containing 0.05% essential oil from basil (EOB); a diet containing 0.05% essential oil from thyme (EOT) and a diet containing 0.05% essential oil from sage (EOS), which are all members of the *Lamiaceae* family. Each experimental diet was composed by first combining the essential oils with the corresponding vegetable oil stock at the compound feed factory and then homogenizing the mixture with the remaining ingredients. Water and feed in mashed form were available ad libitum.

The broiler chicks were reared in an experimental hall with three-tiered Big Dutchman (Vechta, Germany) digestibility cages in accordance with sanitary–veterinary standards (EU 627/15.03.2019). The Cobb 500 Breeder Management Guide was used to establish the microclimate and lighting schedule inside the experimental hall, and a Viper Touch computer was used to automatically monitor them throughout the course of the experiment.

The temperature was manipulated and closely monitored to avoid fluctuations, starting at 32 °C on day 1 and decreasing by 2 °C every week thereafter. The chicks were exposed to 24 h of lighting for the first 4 days and then 23 h of lighting and 1 h of darkness until the end of the experimental period.

Table 1. Experimental diets of starter, grower and finisher phases given to the broiler chickens for 42 days.

Ingredients, % as Feed Basis	Starter 0–12 d	Grower 13–28 d	Finisher I 29–35 d	Finisher II 36–42 d
Corn	45.00	45.00	44.10	44.86
Soybean meal	34.81	24.75	20.77	17.33
Corn gluten	3.00	5.00	5.00	5.00
Wheat	8.86	11.87	15.55	18.51
Vegetal oil	3.42	4.32	5.00	5.46
Alfalfa meal	0.00	5.00	5.00	5.00
Essential oils	0.00	0.05	0.05	0.05
Acidifying	0.00	0.10	0.10	0.10
L—Lysine—HCl	0.26	0.10	0.08	0.11
DL-Methionine	0.30	0.18	0.16	0.14
L-Threonine	0.10	0.10	0.08	0.09
Choline chloride	0.00	0.04	0.04	0.04
Calcium carbonate	1.37	0.96	0.85	0.87
Monocalcium phosphate	1.51	1.33	1.15	1.16
Chloride	0.37	0.35	0.35	0.33
Premix	1.00	1.00	1.00	1.00
Total ingredients	100	100	100	100
Calculated energy and nutrients				
Metabolizable energy, kcal/kg	2975	3025	3100	3150
Crude protein, %	21.41	19.71	18.50	17.50
Ether extract, %	5.34	6.40	7.11	7.58
Ash, %	3.12	3.20	2.99	2.81
Crude fiber, %	3.88	4.43	4.25	4.08
Calcium, %	0.90	0.84	0.76	0.76
Available phosphorus, %	0.45	0.42	0.38	0.38
Total amino acids				
Lysine, %	1.33	1.22	1.11	1.05
Methionine + Cysteine, %	1.00	0.94	0.88	0.84
Threonine, %	0.92	0.81	0.73	0.70
Tryptophan, %	0.23	0.19	0.18	0.16
Valine, %	0.75	0.76	0.76	0.78
Arginine, %	1.07	1.07	1.07	1.08
Isoleucine, %	0.67	0.68	0.69	0.69
Leucine, %	1.10	1.10	1.10	1.10
Determined nutrients				
Polyphenols content, mg GAE/g	2.01	2.43	2.37	2.26
Antioxidant capacity, mM Trolox	14.77	16.98	17.33	17.57

Premix composition per kg feed: 11.000 IU/kg vitamin A; 2.000 IU/kg vitamin D3; 27 IU/kg vitamin E; 3 mg/kg vitamin K; 2 mg/kg vitamin B1; 4 mg/kg vitamin B2; 14.85 mg/kg pantothenic acid; 27 mg/kg nicotinic acid; 3 mg/kg vitamin B6; 0.04 mg/kg vitamin B7; 1 mg/kg vitamin B9; 0.018 mg/kg vitamin B12; 20 mg/kg vitamin C; 80 mg/kg Mn; 80 mg/kg Fe; 5 mg/kg Cu; 0.60 mg/kg Zn; 0.37 mg/kg Co; 1.52 mg/kg I; 0.18 mg/kg Se.

2.2. Production Performance, European Production Efficiency Factor and European Broiler Index

For production performances, the broiler chickens were individually weighed when the actual feeding trial started (12 days) and then on the day of slaughter (42 days). Body weight (BW, g) was recorded, and based on the differences, the average body weight gain (DWG, g) was calculated for the entire experimental period. The feed intake was registered

daily and calculated on a daily basis by subtracting the amount of rejected feed from the offered feed at the beginning and the end of every 24 h (1 day). Based on these recordings, the average daily feed intake was calculated (ADFI, g feed/broiler/day). The feed conversion ratio (FCR, kg feed/kg weight) was calculated. The viability (survival rate, %) of the chickens in each group was monitored during the experimental period. The relative growth rate (RGR) was calculated using Equation (1), where W1 represents the initial live weight, and W2 represents the live weight at the end of the experimental period. After calculation of the viability percentage and FCR, the European Production Efficiency Factor (EPEF) and the European Broiler Index (EBI) were calculated with Equations (2) and (3), which allow for the measurement of the broilers' performances [19].

$$\text{RGR} = \frac{\text{W2} - \text{W1}}{0.5(\text{W1} + \text{W2})} \times 100 \quad (1)$$

$$\text{EPEF} = \frac{\text{viability} \times \text{body weight}}{\text{age} \times \text{feed conversion ratio}} \times 100 \quad (2)$$

$$\text{EBI} = \frac{\text{viability} \times \text{daily weight gain}}{\text{feed conversion ratio} \times 10} \quad (3)$$

2.3. Slaughter Measurements, Sampling of Meat and Intestinal Content

At the end of the experimental period when the birds were 42 days old, 24 broiler chickens (6 chickens/group) with similar body weights were selected to be slaughtered as presented elsewhere [8]. The defeathered carcass without the head and feet was eviscerated manually and weighed as the carcass weight. After evisceration, the carcass was dissected, and the anatomical parts with commercial interest were measured (thigh muscle, breast muscle, liver, gizzard and heart) and expressed as percent (%) of the BW. Both upper parts of the thigh meat without skin were collected and divided into two parts. One part was sampled and then vacuum-packed into plastic bags and stored at −20 °C until chemical analyses were performed; the other part was sent to the laboratory for colorimetric and textural analyses.

The intestinal content was aseptically collected from the small intestine and caecum sections in sterile plastic tubes and quickly transferred on ice to the laboratory. One gram of each intestinal content segment (small intestine and caecum) was homogenized with 7 mL Brain Heart Infusion (BHI) broth (Oxoid LTD, England CM1135) supplemented with 2 mL sterile glycerol and immediately frozen at −20 °C until the microbiological analyses of *Lactobacillus* spp., *Staphylococcus* spp., *Escherichia coli*—β-hemolytic, *Enterobacteriaceae*, *Clostridium* spp., *Coliforms*, *Enterococcus* spp. and *Salmonella* spp. were performed.

2.4. Chemical Analyses

A spectrophotometric method was used for total polyphenols determination based on the color reaction between the specific reagent Folin Ciocalteu and the methanolic extract of the meat samples and quantification at 732 nm against gallic acid used as the standard for the calibration curve. The results were given in milligrams of gallic acid equivalents per gram of material (mg GAE/g).

The DPPH reagent was used in order to estimate the antioxidant capacity with the absorbance being registered at 517 nm against Trolox, which was used as a standard for the calibration curve. The results were given in millimoles per liter of solution (mM Trolox). Both polyphenols content and antioxidant capacity were determined using a V 530 Jasco (Japan Servo Co. Ltd., Tokyo, Japan) spectrophotometer, and the methods are detailed elsewhere [20].

The lipid composition of the meat samples was determined by fatty acids transformation into methyl esters, injection of the sample solution into the chromatographic column and identification based on retention time and fatty acids standards. The apparatus used for determination was a gas chromatograph Perkin-Elmer Clarus 500 (Waltham, MA, USA)

equipped with a flame ionization detector and capillary separation column with a high polar stationary phase TRACE TR-Fame (Thermo Electron, Waltham, MA, USA) with dimensions of 60 m × 0.25 mm × 0.25 µm, as described elsewhere [21]. The average amount of each fatty acid (FA) was used to calculate the sum of the total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

2.5. Colorimetric and Textural Parameters Analyses

The thigh meat color determination was performed using a Konica Minolta CR-400 (Holdings Inc., Tokyo, Japan) colorimeter, which was calibrated using the standard white ceramic reference (illuminate C). The lightness (L *; dark to light on a scale of 0 to 100), redness (a *; green to red on a scale of −60 to +60) and yellowness (b *; blue to yellow on a scale of −60 to +60) were determined according to the CIE Lab (CIE, Commission International de l'Eclairage, Vienna, Austria) trichromatic system from the average of 3 measurements/sample (18 measurements/group). The hue angle (H°), chroma (C *) and color difference (ΔE *) psychometric color parameters were determined with appropriate formulas [22]. The meat color readings were taken in an area free of obvious color defects that may affect a uniform color reading.

The textural parameters (hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess and chewiness) of the thigh meat samples were determined with a CT3 Texture Analyzer, AMETEK Brookfield (Australia Pty Ltd., Melbourne, Australia), and a software package as described elsewhere [22]. Briefly, a double compression cycle test was performed up to 50% compression of the original portion height with a glass cylinder of 20 mm diameter followed by a time of 5 s to allow the elapse between the two compression cycles. Force–time deformation curves were obtained with a 10 kg load cell applied at a crosshead speed of 2 mm/s, which was applied three times for each sample (18 measurements/group).

2.6. Microbiota Analyses

After the samples defrosted, decimal dilutions in phosphate-buffered saline pH 7.0 (PBS, Dulbecco A; Oxoid Livingstone Ltd., London, UK) were performed for the enumeration of microbial populations and assessed for analyses of lactic acid bacteria as reported elsewhere [1]. Briefly, *Lactobacillus* spp. counts were determined by plating on Man, Rogosa and Sharpe agar selective medium (Oxoid CM0361). *Escherichia coli* biotype β-hemolytic was determined by inoculating 0.01 mL from a 10^{-1} dilution on sheep blood agar [Trypticase soy agar (TSA) 5% (*w*/*v*)] and incubated at 37 °C for 24 h in aerobic conditions. Coagulase-positive *Staphylococcus* spp. was enumerated on Baird–Parker agar (Oxoid CM0275) supplemented with egg yolk tellurite emulsion at 37 °C for 48 h in aerobic conditions. *Clostridium* spp. were cultured on reinforced clostridial agar (Oxoid CM0151) at 37 °C for 48 h in anaerobic conditions (Oxoid jar with Anaerogen 2.5 L). Enumeration of *Enterococcus* spp. was performed on Slanetz–Bartley agar (Oxoid CM0377) followed by incubation in anaerobic conditions at 37 °C for 48 h. The presence of *Coliforms* was determined on MacConkey agar (Oxoid CM0007) in aerobic conditions at 37 °C for 24 h. *Salmonella* spp. was evaluated on *Salmonella-Shigella* agar (Oxoid CM0099) in aerobic conditions at 37 °C for 24 h as described by Dumitru et al. [23]. Every sample was repeated three times, and the microbiota enumerations were expressed as Log10 CFU per gram.

2.7. Statistical Analysis

The graphs and statistical interpretation of the results obtained were made in GraphPad Prism software, version 13.2 (GraphPad Software, La Jolla, CA, USA). The effect of the essential oils on production performances and meat quality was carried out using one-way analysis of variance (ANOVA), and the values were determined to be significant (when * $p < 0.05$ and ** $p < 0.001$) or highly significant (*** $p < 0.0001$) between groups. Tukey's multiple range tests were used to determine the significance of individual mean differences at $p < 0.05$.

3. Results

3.1. Effect of Dietary Supplements on Production Performances, Production Efficiency Factors and Anatomical Parts Development in Broiler Chickens Supplemented with Essential Oils

The effects of the studied EO on production performances, efficiency factors and anatomical parts development are presented in Table 2. Final BW and DWG were improved ($p < 0.05$) in the experimental groups compared with the control. The FCR was ($p < 0.05$) lower in the experimental groups when compared to the control. This led to better ($p < 0.05$) production efficiency factors (EBI and EPEF) and carcass weight but without significant influence in anatomical parts development.

Table 2. Effect of dietary supplements on production performances, efficiency factors and organ development in broiler chickens supplemented with essential oils.

Item	CON	EOB	EOT	EOS	SEM	*p*-Value
Production performances						
Initial BW, g	246.8	246.6	247.4	247.5	3.480	0.9997
Final BW, g	2676 ^{b}	2784 ^{a}	2799 ^{a}	2791 ^{a}	16.98	0.0285
DWG, g/day	86.76 ^{b}	92.51 ^{a}	93.42 ^{a}	95.09 ^{a}	0.709	0.0494
ADFI, g/chick/day	135.9	132.2	129.9	130.6	3.429	0.9265
FCR, kg feed/kg weight	1.62 ^{a}	1.55 ^{b}	1.55 ^{b}	1.53 ^{b}	0.029	0.0317
Viability, %	94.20	100	100	100	-	-
RGR, %	166.2	167.5	167.5	167.4	3.280	0.0577
Efficiency factors, %						
EBI	536.9 ^{b}	565.4 ^{a}	568.9 ^{a}	570.7 ^{a}	0.065	0.0200
EPEF	591.4 ^{b}	622.1 ^{a}	624.1 ^{a}	628.0 ^{a}	5.364	0.0090
Anatomical parts development, %						
Carcass	78.95 ^{b}	83.05 ^{a}	82.57 ^{a}	83.14 ^{a}	0.483	0.0257
Thigh muscle	17.32	17.74	17.59	18.64	0.253	0.2856
Breast muscle	21.65	22.09	22.21	21.99	0.191	0.3715
Liver	2.13	2.13	2.27	2.30	0.051	0.2957
Gizzard	1.15	1.16	1.17	1.17	0.023	0.9511
Heart	0.53	0.54	0.53	0.54	0.013	0.9917

$^{a, b}$ Values with different superscripts in the same row differ significantly ($p < 0.05$). CON—control diet; EOB—diet containing 0.05% essential oil of basil; EOT—diet containing 0.05% essential oil of thyme; EOS—diet containing 0.05% essential oil of sage; SEM—standard error of the mean; BW—body weight; DWG—daily weight gain; ADFI—average daily feed intake; FCR—feed conversion ratio; RGR—relative growth rate; EBI—European Broiler Index; EPEF—European Production Efficiency Factor.

3.2. Effect of Dietary Supplements on Colorimetric and Textural Parameters of Thigh Meat

The effects of basil, thyme and sage EO as PFA on colorimetric and textural parameters are shown in Table 3. Thigh meat lightness (L *) was significantly higher in the EO-supplemented groups as well as hue angle (H°) compared with the CON samples. The textural parameters were not influenced by the dietary supplements ($p > 0.05$).

Table 3. Effect of dietary supplements on colorimetric and textural parameters.

Item	CON	EOB	EOT	EOS	SEM	*p*-Value
Colorimetric parameters						
Lightness (L *)	42.38 ^{b}	46.21 ^{a}	47.18 ^{a}	45.62 ^{a}	0.285	0.0233
Redness (a *)	2.79	2.88	2.91	2.92	0.519	0.0520
Yellowness (b *)	9.31	9.68	9.54	9.67	0.188	0.0671
Chroma (C)	10.22	10.67	10.51	10.73	0.102	0.2941
Hue angle (H°)	4.06 ^{b}	5.21 ^{a}	5.37 ^{a}	5.26 ^{a}	0.223	0.0394
Color difference (ΔE *)	0.53	0.88	0.92	0.89	0.372	0.0607

Table 3. *Cont.*

Item	CON	EOB	EOT	EOS	SEM	*p*-Value
Textural parameters						
Hardness, grams	3194	3283	3213	3228	15.27	0.9963
Gumminess, grams	1391	1379	1315	1369	12.30	0.2811
Springiness, millimeters	2.50	2.65	2.59	2.57	0.132	0.1155
Resilience	2.39	2.65	2.22	2.14	0.015	0.2348
Cohesiveness	0.38	0.35	0.33	0.34	0.113	0.0874
Adhesiveness, Megajoule	0.22	0.27	0.30	0.29	0.047	1.0455
Chewiness, Megajoule	15.12	16.07	14.33	13.98	4.886	0.9999

[a, b] Values with different superscripts in the same row differ significantly ($p < 0.05$). CON—control diet; EOB—diet containing 0.05% essential oil of basil; EOT—diet containing 0.05% essential oil of thyme; EOS—diet containing 0.05% essential oil of sage; SEM—standard error of the mean.

3.3. Effect of Dietary Supplements on Antioxidant Capacity and Polyphenols Content in Thigh Meat

The bioactive compounds with antioxidant potential determined in the meat samples are presented in Figure 1. The results revealed that the diets supplemented with EO led to a significant increase in total antioxidant capacity ($p = 0.0144$) (Figure 1A) and total polyphenols content ($p = 0.0003$) (Figure 1B) in the meat samples compared with the control samples.

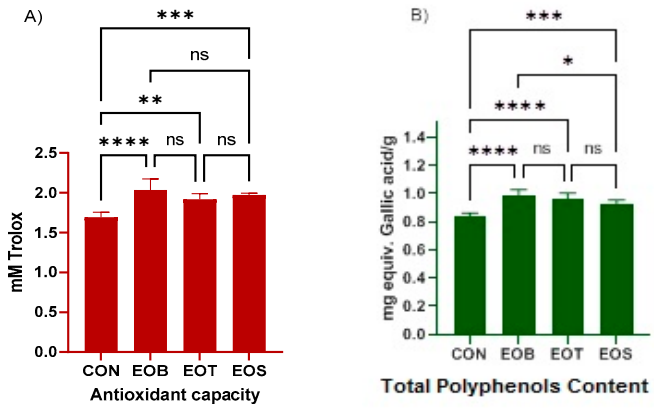

Figure 1. Effect of dietary EO supplement on (**A**) antioxidant capacity and (**B**) total polyphenols content determined in thigh meat samples. CON—control diet; EOB—diet containing 0.05% essential oil of basil; EOT—diet containing 0.05% essential oil of thyme; EOS—diet containing 0.05% essential oil of sage; ns—not significant. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, **** $p < 0.00001$.

3.4. Effect of Dietary Supplements on Fatty Acid Composition of Thigh Meat

Table 4 shows the lipid content determined in the meat samples. The sum of SFA and MUFA was significantly lower ($p < 0.05$) in the EO-supplemented groups compared with the CON group. The sum of PUFA was significantly increased in the EOB, EOT and EOS samples compared with the CON samples. From the n-6 PUFA, linoleic acid (LNA) was the most abundant in the experimental meat samples, while from the n-3 PUFA, alpha-linolenic and eicosapentaenoic acids were significantly increased compared with the control samples. Concomitantly the n-6/n-3 ratio decreased in all the experimental samples compared with the control samples.

Table 4. Effect of dietary supplements on the fatty acid composition of thigh meat samples.

Fatty Acids, g/100 g	CON	EOB	EOT	EOS	SEM	*p*-Value
Butyric C4:0	0.55 ^{a}	0.39 ^{b}	0.42 ^{b}	0.41 ^{b}	0.018	0.0009
Caproic C6:0	0.46 ^{a}	0.31 ^{b}	0.30 ^{b}	0.32 ^{b}	0.018	0.0001
Caprylic C8:0	0.65 ^{a}	0.16 ^{b}	0.16 ^{b}	0.18 ^{b}	0.057	<0.0001
Capric C10:0	0.42 ^{a}	0.06 ^{b}	0.06 ^{b}	0.10 ^{b}	0.027	<0.0001
Lauric C12:0	0.02	0.03	0.03	0.03	0.004	0.4358
Myristic C14:0	1.35 ^{a}	0.57 ^{c}	0.71 ^{bc}	0.78 ^{b}	0.067	<0.0001
Myristioleic C14:1	0.10	0.11	0.11	0.12	0.003	0.1672
Pentadecanoic C15:0	0.47	0.49	0.51	0.47	0.122	0.0719
Pentadecenoic C15:1	1.38 ^{b}	1.73 ^{a}	1.67 ^{a}	1.76 ^{a}	0.064	0.0326
Palmitic C16:0	25.76 ^{a}	23.97 ^{c}	24.77 ^{b}	24.67 ^{b}	0.148	<0.0001
Palmitoleic C16:1	2.48	2.45	2.50	2.48	0.077	0.0513
Heptadecanoic C17:0	0.08	0.09	0.09	0.12	0.006	0.0765
Heptadecenoic C17:1	0.29	0.31	0.30	0.31	0.022	0.1346
Stearic C18:0	10.07	10.52	10.48	10.54	0.055	0.0806
Oleic cis C18:1n9	42.01 ^{a}	40.06 ^{b}	39.44 ^{b}	39.97 ^{b}	0.245	0.0293
Linoleic cis C18:2n6	7.48 ^{b}	8.04 ^{a}	8.11 ^{a}	8.13 ^{a}	0.060	<0.0001
Linolenic γ C18:3n6	0.06	0.03	0.07	0.06	0.007	0.3791
Linolenic α C18:3n3	0.10 b	0.24 ^{a}	0.23 ^{a}	0.24 ^{a}	0.004	0.0376
Conjugated LA C18:2	0.33	0.32	0.32	0.31	0.004	0.4257
Octadecatetraenoic C18:4n3	1.38 ^{b}	1.51 ^{ab}	1.60 ^{ab}	1.76 ^{a}	0.041	0.0450
Eicosadienoic C20:2n6	2.51	2.85	2.75	2.68	0.087	0.9057
Arachidonic C22:4n6	0.15	0.12	0.09	0.12	0.006	0.0921
Docosadienoic C22:2n6	0.57	0.64	0.59	0.61	0.017	0.1796
Docosatrienoic C22:3n6	0.64	0.77	0.63	0.71	0.028	0.1485
Eicosapentaenoic C20:5n3	0.71 ^{c}	0.80 ^{b}	0.81 ^{b}	0.94 ^{a}	0.020	<0.0001
Lignoceric C 24:0	0.80 ^{b}	0.99 ^{a}	0.86 ^{ab}	0.86 ^{ab}	0.115	0.0612
ΣSFA	40.40 ^{a}	37.68 ^{b}	38.45 ^{b}	38.54 ^{b}	0.486	0.0335
ΣMUFA	46.26 ^{a}	44.67 ^{b}	44.02 ^{b}	44.63 ^{b}	0.190	0.0337
ΣPUFA	13.70 ^{b}	15.08 ^{a}	15.11 ^{a}	15.43 ^{a}	0.172	0.0002
Σn-6 PUFA	11.70 ^{b}	12.52 ^{a}	12.47 ^{a}	12.49 ^{a}	0.138	0.0085
Σn-3 PUFA	2.00 ^{b}	2.56 ^{a}	2.64 ^{a}	2.94 ^{a}	0.092	0.0063
n-6/n-3 ratio	6.76 ^{a}	4.83 ^{b}	4.72 ^{b}	4.29 ^{b}	0.428	0.0068

$^{a, b, c}$ Values with different superscripts in the same row differ significantly ($p < 0.05$). CON—control diet; EOB—diet containing 0.05% essential oil of basil; EOT—diet containing 0.05% essential oil of thyme; EOS—diet containing 0.05% essential oil of sage; SEM—standard error of the mean; ΣSFA—saturated fatty acids; ΣMUFA—monounsaturated fatty acids; ΣPUFA—polyunsaturated fatty acids.

3.5. Effect of Dietary Supplements on Intestinal Microbiota of Broiler Chickens

The effect of EO on the broiler chickens' intestinal health is presented in Figure 2. The results revealed that the *Lactobacillus* spp. significantly increased in both the intestine and caecum. The *Enterococcus* spp. was significantly lowered only in the EOS group in the caecum segment. Although the Coliforms were decreased in the intestinal segment, a significantly lower colony number was observed only in the caecum of the broilers supplemented with EO compared with the CON. *Clostridium* spp. was also modified by the addition of EO to the experimental diets.

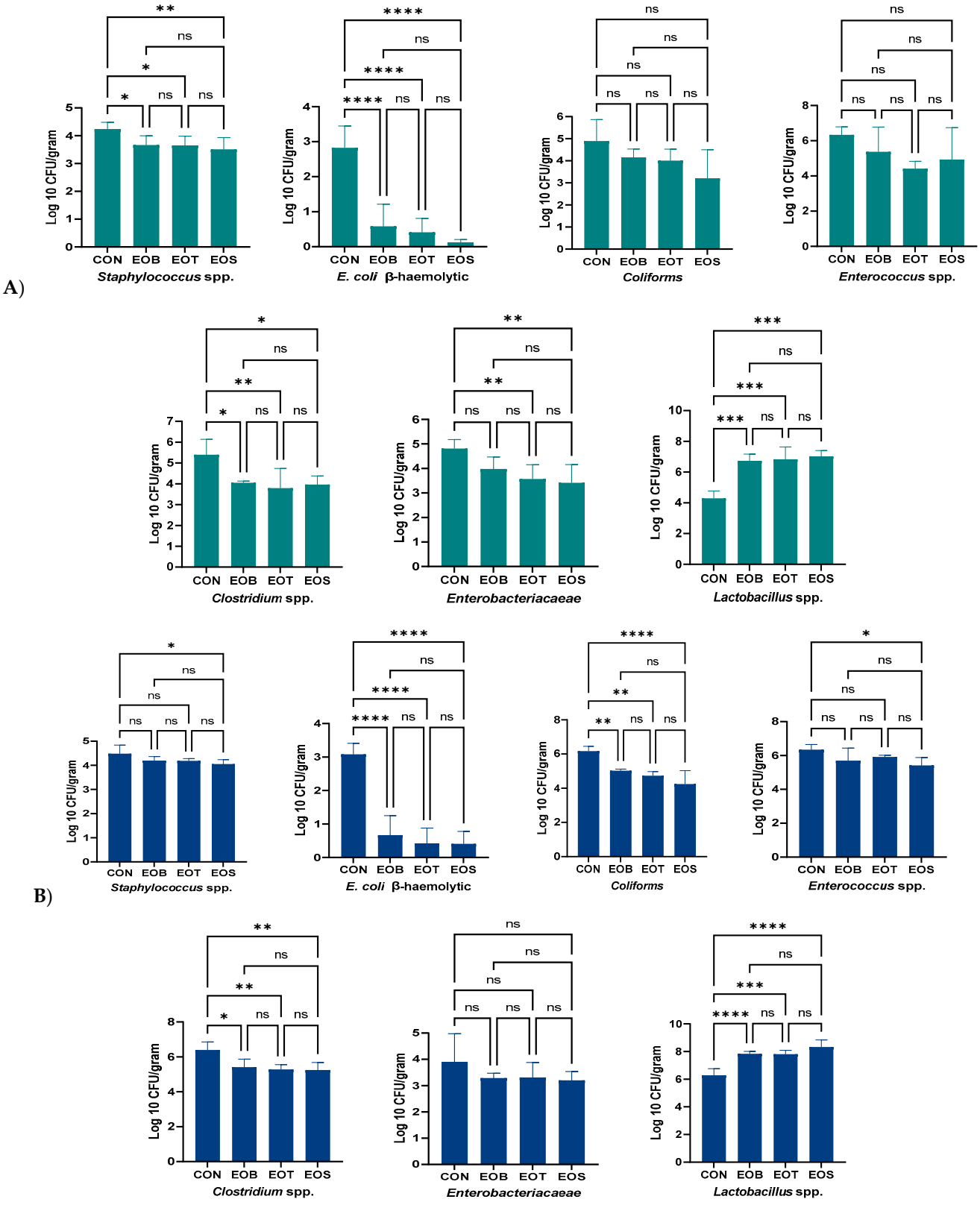

Figure 2. Effect of dietary EO supplements on the intestinal microbiota of broiler chickens determined from (**A**) small intestine segment and (**B**) caecum segment. The results are expressed as (Log10 CFU/g). CON—control diet; EOB—diet containing 0.05% essential oil of basil; EOT—diet containing 0.05% essential oil of thyme; EOS—diet containing 0.05% essential oil of sage. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, **** $p < 0.00001$. ns—not significant.

4. Discussion

In the current study, utilization of EO from basil, thyme and sage resulted in significant improvements in broiler performances, especially for BW, DWG, FCR, production efficiency factors and carcass percentage compared with the CON group. These observed improvements might be attributed to the antimicrobial and antioxidant activities of the EO used, which have the potential to improve gut health and stimulate digestive enzymes, resulting in increased digestion of dietary nutrients for growth [24]. Previous studies in which EO were used as antibiotic replacements to promote growth, improve the FCR and reduce feed costs, reported differentiated results. While some studies observed positive effects [25–27], others did not show differences in comparison to the control [28,29]. The effects of EO utilization on broilers' production are in line with available findings on these parameters, demonstrating that the usage of EO, such as garlic, thyme, spearmint, black cumin, sage, clove and lemongrass, is economically profitable [5,30,31]. Compared to the EO-supplemented groups, the lower viability rate in the CON group might be attributed to the low anti-inflammatory and antioxidant properties as recently reported [32]. Similar to this study, increased carcass percentage and no effect of EO on commercial parts (thigh muscle, breast muscle, liver, gizzard, heart) have been reported [33,34]. The differences observed among the various studies in response to EO supplementation can be attributed to the dose and form of EO used, dietary composition and hygienic and environmental conditions. However, further research is needed to better understand how these mechanisms are responsible for improving chickens' production performances, or maybe, a combination of multiple mechanisms is involved in this process.

The colorimetric parameters of the thigh meat reported in Table 3 showed significant differences between the CON samples and the EOB, EOT and EOS samples for the L * and H * parameters. The other parameters were not different ($p > 0.05$), although generally, the EO-supplemented groups presented higher values for redness (a *) and yellowness (b *) than the CON samples. This effect is beneficial from the consumers' point of view because meat color is an important factor and has an impact on the first impression of the buyers [27]. Furthermore, meat oxidation is strongly related to lower a * values or myoglobin denaturation, which could negatively affect the meat quality. Other studies [35–37] reported that different PFA influenced meat color differently in terms of L *, a * and b * parameters. However, these discrepancies between the studies and data reported in the literature are given by the factors, such as diets, age, genetics and muscle parts, analyzed (breast, leg and thigh). Since the thigh part has a high content of red muscle fibers compared to the breast muscle, which has only white muscle fibers, we considered that these differences are caused by the myoglobin presence in the thigh muscles. Together with color, meat texture is also considered an important meat quality factor for consumers' preferences. The texture analysis of broiler thigh meat showed no significant effect ($p > 0.05$), which indicates that supplementation with EO in broiler diets did not influence the studied parameters. Similar results were reported previously when different plant oil extracts were fed to broilers [28,35].

In terms of antioxidant potential, as mentioned above, EO can exhibit a significant antioxidant effect when added to broilers' feeds. This effect was confirmed, and it was reflected in the increased antioxidant capacity and polyphenols content in the meat samples of the EOB-, EOT- and EOS-supplemented groups compared with those from the CON group, as presented in Figure 1. Due to high concentrations of PUFA, the chicken's meat is prone to oxidative deterioration; however, the studied EO can retard oxidant degradation in the thigh meat. Previous in vitro studies reported that utilization of EO derived from the *Lamiaceae* family (oregano, basil, sage, rosemary, thyme) exhibited significant antioxidant activities in broiler meat [10,37,38], minced pork [39] or ground beef [40]. This effect is beneficial because the antioxidant activity derived from the phenolic hydroxyl group interacts with peroxy radicals during the initial process of lipid oxidation and, subsequently, retards the hydroxyl peroxide formation [41]. Although the majority of EO derived from *Lamiaceae* family plants possess antioxidant properties with a clear correlation between the

inhibition of hydroperoxide formation and bioactive compounds (thymol, linalool, eugenol, camphor, etc.), it is still unclear to what extent the antioxidant activity is attributed only to the EO; however, compared to the mentioned studies, our results showed a significant increase in terms of bioactive compounds with antioxidant potential.

The effect of EO on the thigh meat fatty acids composition showed a significant decrease in the total SFA and MUFA and a significant increase in the total of n-6 and n-3 PUFA in the EOB, EOT and EOS samples compared with the CON samples. From the n-6 PUFA family, LNA was the most abundant ($p < 0.0001$) in the experimental samples, whereas from the n-3 PUFA, a significantly ($p < 0.05$) higher concentration of eicosapentaenoic acid, which is essential for human health, was determined. The increased percentage of n-6 and n-3 PUFA in the chicken thigh meat led to a significantly lower n-6/n-3 ratio in the experimental samples compared with those from the control. Similarly, it was recently reported that the dietary addition of thyme, lavender, eucalyptus or bergamot EO increased the percentages of essential n-3 and n-6 PUFA, concomitant with a decreased n-6/n-3 ratio [42,43]. A similar observation was previously reported when *Lamiaceae* plants were used in broiler diets [5]. These effects have not been properly investigated, however. As mentioned above, we considered that these supplements acted as effective free radical scavengers and also influenced the endogenous antioxidant defense systems in the animal [44]. Unfortunately, there are scarce studies on the effect of basil, thyme and sage essential oils on the fatty acid profile of thigh meat, which leaves the door open for further investigations on this aspect.

The intestinal microbiota is an important factor in avian health and poultry production. Broiler chickens during the first days are very susceptible to infectious pathogenic microorganisms, especially *Escherichia coli* and *Salmonella*. The integrity of intestinal microbiota is also crucial for consumer safety, since gut contents may contaminate the carcass with a variety of pathogens, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, *Campylobacter* and *Salmonella* [45]. Although the pathogenic microorganisms compete with the host for nutrients in the small intestine, the qualitative and quantitative composition of intestinal microbiota may be affected by many factors (environmental and housing conditions, microclimate and feed composition), which can result in necrotic lesions in the intestinal wall [1,46]. This study revealed that EO derived from basil, thyme and sage used in broiler chickens had a bacteriostatic effect, reducing the CFU of *Escherichia coli* and *Clostridium* spp. bacteria in the small intestine and caecum. Additionally, the same PFA increased the CFU of *Lactobacillus* spp., which are beneficial bacteria. Although the effect on *Enterococcus* spp., *Coliforms*, *Staphylococcus* spp. and *Enterobacteriaceae* was different in the intestinal and caecal segments, the effect was noted on both parts. Since the small intestine is responsible for the digestion and absorption of ingested feed, its structure is assumed to be related to its function and is affected differently compared with the caecum segment [47]. The lower harmful bacteria, especially *Escherichia coli*, and the increased lactobacilli count found in this study are in line with the findings of others [48–51] who reported a lower CFU of *Escherichia coli* in broilers fed different EO as PFA and an improved proliferation of *Lactobacillus* spp. count in intestinal and caecum segments. Compared with other PFA reported in the literature, EO, studied as complex mixtures, may exhibit antimicrobial activities that differ from those of their major component studied alone. As a main mechanism, EO have an impact on stabilizing feed hygiene, which affects the ecosystem of the intestinal microorganisms by reducing the number of unwanted bacteria. Compared with other studies, these results indicate that EO from basil, thyme and sage may represent good dietary PFA for broiler chickens, which is particularly important due to the increasing resistance of *Escherichia coli* to antimicrobials.

5. Conclusions

Essential oils derived from basil, thyme and sage plants at a 0.05% inclusion level can be used efficiently as phytogenic feed additives in broiler chicken diets during grower–finisher phases. They improved the production performances, antioxidant capacity and

concentration of polyunsaturated fatty acids in thigh meat. The intestinal microbiota of the chickens was influenced by increasing the beneficial bacteria (lactobacilli) in the intestine and caecum and decreasing the harmful bacteria, especially *Staphylococcus* spp. and *Escherichia coli*. From the studied supplements, the EOS diet was the most effective in terms of improving the intestinal microbiota, while the EOB diet was more effective at increasing the antioxidant capacity and polyphenols content in the meat.

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Institutional Review Board Statement: All procedures concerning animals' care, handling and sampling were conducted under the approval of the ethical committee of the institute according to the Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, Law 43/11.04.2014, Directive 2010/63/EU) before the initiation of the study and followed the Romanian guidelines. The experimental procedures were approved by the Ethical Commission of the National Research and Development Institute for Biology and Animal Nutrition no. 5121/12.08.2020.

Data Availability Statement: Data is contained within the article.

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