Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: In vitro antimicrobial activities and effects on growth performance

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ABSTRACT: The present study was conducted to characterize the in vitro antimicrobial activities of 3 essential oils [oregano, rosemary, and a commercial blend of essential oils (BEO)] against pathogenic and nonpathogenic bacteria and to evaluate their effects on broiler chicken performances. The chemical composition of the essential oils was determined using the gas chromatography interfaced with a mass spectroscopy. The disc diffusion method, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) were applied for the determination of antimicrobial activities of essential oils. In vivo study, a total of seven hundred fifty 1-d-old male broiler chickens were assigned to 6 dietary treatment groups: basal diet (control; CON), CON + 44 mg of avilamycin/kg (A), CON + 100 mg of rosemary essential oil/kg (ROS), CON + 100 mg of oregano essential oil/kg (OR), CON + 50 mg of rosemary and 50 mg of oregano essential oils/kg (RO), and CON + 1,000 mg of BEO/kg (essential oil mixture, EOM). The essential oils isolated from rosemary and oregano were characterized by their greater content of 1,8-cineole (49.99%) and carvacrol (69.55%), respectively. The BEO was mainly represented by the aldehyde (cinnamaldehyde) and the monoterpene (1,8-cineole) chemical groups. The results of the disc diffusion method indicated that the rosemary essential oil had antibacterial activity \((P \leq 0.05)\) against only 3 pathogenic bacteria, Escherichia coli (8 mm), Salmonella indiana (11 mm), and Listeria innocua (9 mm). The essential oil of oregano had antimicrobial activities \((P \leq 0.05)\) on the same bacteria as rosemary but also on Staphylococcus aureus (22 mm) and Bacillus subtilis (12 mm). Oregano essential oil had greater \((P \leq 0.05)\) antimicrobial activities against pathogenic bacteria than rosemary essential oil but they had no synergism between them. The BEO showed an increased antimicrobial activity \((P \leq 0.05)\) against all studied bacteria (pathogenic and nonpathogenic bacteria) except for Lactobacillus rhamnosus. The supplementation of the basal diet with avilamycin or essential oils improved \((P \leq 0.05)\) broiler chicken BW, BW gain, and G:F compared with the CON diet. There were no differences in growth performances among birds fed A, ROS, OR, RO, or EOM diets. In general, essential oils contained in rosemary, oregano, and BEO can substitute for growth promoter antibiotics. Although the 3 essential oils had different antimicrobial activities, they exhibited the same efficiency in broiler chickens.

Key words: antimicrobial activity, broiler chicken, essential oil, growth performance, oregano, rosemary
INTRODUCTION

Components of essential oils have shown biological properties such as antioxidant and antimicrobial activities (Jang et al., 2007; Windisch et al., 2008). In vitro antimicrobial activity of essential oils from the Labiatae family such as oregano and rosemary have been reported (Sivropoulou et al., 1996; Burt and Reinders, 2003). Helander et al. (1998) and Faleiro et al. (1997) showed antimicrobial properties against only pathogenic bacteria. Oussalah et al. (2006) and Celiktas et al. (2007) reported that oregano and rosemary essential oils were active against pathogenic bacterial species such as Escherichia coli and Staphylococcus aureus. Lee and Ahn (1998) observed that the Cinnamomum cassia essential oil exhibited growth-inhibiting activity against nonpathogenic bacteria. However, to our knowledge, there are few data, in vitro, on the antimicrobial activities of oregano and rosemary essential oils against nonpathogenic bacteria.

The effect of rosemary and oregano essential oils on broiler growth performances has not been well documented (Botsoglou et al., 2002, 2004; Hernández et al., 2004). Several studies indicated that the use of essential oils improved broiler feed conversion ratio (Hertrampf, 2001; Windisch et al., 2008). However, most data available in literature are obtained from commercial products containing blends of different essential oils. Thus, it is difficult to evaluate the efficiency of each essential oil in terms of its botanical origin and active compounds. In addition, we have no information about the relationship between in vitro antimicrobial potential and efficiency of essential oils in broiler chickens. Perhaps essential oils, which inhibit pathogenic and nonpathogenic bacteria, are more efficient in broiler chickens. The objective of the present study was to evaluate the antimicrobial activities of 3 essential oils (oregano, rosemary, and a blend of essential oils) against pathogenic and nonpathogenic bacteria and their effects on broiler chicken performances.

MATERIALS AND METHODS

The animal experiment was conducted in accordance with the principles and specific guidelines presented in decree number 87-848 of October 19, 1987, from the French Rural Code on experiments conducted with animals.

Resources of Essential Oils

Three essential oils were used in the present study: rosemary (Rosmarinus officinalis), oregano (Origanum vulgare), and a blend of essential oils. Rosemary and oregano plants were collected (Hadjeb Elayoun, Tunisia), dried, and subjected to water distillation according to the method of Zaouali et al. (2010). The 3 essential oils preparations were stored at 4°C in dark glass bottles until assays. The essential oils of rosemary and oregano were provided by a commercial company (Carthago, Sousse, Tunisia), and the blend of essential oils (BEO) was provided by another commercial company (Phytosynthése, Riom, France).

Composition of the Essential Oils

The composition of the essential oils were determined using gas chromatography (GC; Agilent 6890N, Agilent Technologies, Paris, France) interfaced with mass spectroscopy (MS; Agilent 5973N, Agilent Technologies). The capillary column used was the HP5-MS 5% phenyl methyl siloxan (length: 30 m; internal diameter: 0.25 mm; film thickness: 0.25 µm) and an automatic passer (Agilent 7683B; Agilent Technologies). Helium was the carrier gas at a flow rate of 1 mL/min. The column temperature was initially adjusted at 5°C (during 1 min) then increased progressively at a rate of 2°C/min to reach 300°C within 130 min. The samples were diluted in ethanol (1/10) then 1 µL was injected into GC-MS (Bampidis et al., 2005). The components were identified by comparing their relative retention times and mass spectra with the standard data (NIST 05, Mass Spectra Library, National Institute of Standards and Technology, Gaithersburg, MD). The GC-MS analyses were conducted at Institut National de la Recherche et d’Analyses Physico-chimiques (Sidi-Thabet, Tunisia).

In Vitro Study

Microbial Strains. Essential oils were individually tested against a panel of microorganisms including lactic acid bacteria and undesirable bacteria. Microorganisms were provided from the culture collection of the Laboratory of Microbial Technology and Ecology at Institut National des Sciences Appliquées et de Technologie (Tunis, Tunisia) and UPR-000263 JE 1 at Ecole Nationale des Ingénieurs en Techniques Agricoles (Bordeaux, France). The lactic acid bacteria were Lactobacillus plantarum (T25), Lactobacillus rhamnosus (TB1), Lactobacillus reuteri (J1), Enterococcus faecium (T10), Enterococcus fecalis (T8), and Lactobacillus salivarius (Ecole Nationale des Ingénieurs en Techniques Agricoles). The undesirable bacteria were E. coli 011 (CIP), Salmonella indiana (CIP), Listeria innocua 8011T (CIP), Bacillus subtilis 168 (CIP), and S. aureus 20256 (CIP). Trypticase soy broth (TSB) and de Man-Rogosa-Sharpe (MRS) broth (Pronadisa, Madrid, Spain) were used, respectively, for growing and diluting undesirable and lactic acid bacterial cultures. Undesirable strains were cultured overnight at 37°C in TSB (Pronadisa), and lactic acid bacteria were cultured overnight at 37°C in MRS (Pronadisa).

Disk Diffusion Method. Antibacterial activities of the 2 essential oils and the BEO were assessed using the paper disk agar diffusion method according to Sacchetti et al. (2005) and Rasooli et al. (2006). Each tested microorganism was set up 16 h before the as-
says to reach the log phase of growth (optic density at a wavelength of 600 nm = 0.4 to 0.5). Molten agar (5 mL, 40 to 45°C) containing 0.1 mL of microorganism suspension (10^2 cfu/mL) was spread over the surface of agar plates containing appropriate medium of each microorganism and left to solidify. Absorbent disks (Whatman disk No. 3 of 6-mm diameter) were placed in the inoculated Petri dishes than impregnated with 10 µL of different essential oils. The blend of rosemary and oregano was used to study the effect of the one-half of the amount of each compound (vol/vol) from each essential oil on growth bacteria. Before incubation, all the Petri dishes were kept in a refrigerator (4°C) for 2 h to stop the bacteria from multiplication. Then they were incubated at 37°C for 24 h and the diameters of the inhibition zones, including the 6-mm disk, were measured (mm). Ethanol and chloramphenicol (Bio-mérieux, Craponne, France) were used as negative and positive control standards, respectively, to determine the sensitivity of each bacterial species tested. All the tests were performed in triplicate.

**Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Determination**

The minimum inhibitory concentration (MIC) values were determined for undesirable bacterial strains, which were sensitive to the essential oils in the disk diffusion assay. The inocula of the bacterial strains were prepared from 16-h broth cultures. The molten agar (5 mL) containing 100 µL of bacterial suspension (10^2 cfu/mL) was poured into sterile Petri plates containing the appropriate medium of each microorganism and left to solidify. Serial dilutions (1/2, 1/4, 1/8, and 1/10) of each essential oil were made with ethanol and spread (10 µL) over the sterile paper disks (Whatman disk No. 3 of 6 mm diameter). Petri dishes were incubated at 37°C for 24 h. Ethanol served as the negative control. The MIC was calculated as the least concentration of essential oil showing complete inhibition of the tested microorganism.

The minimum bactericidal concentration (MBC) was determined as the least concentration of essential oils showing complete absence of bacterial growth. In this assay, 50 µL from each dilution of essential oil showing growth inhibition zone in disc diffusion method was added to 5 mL of TSB tubes containing 10^2 cfu/mL. The tubes were then incubated at 37°C for 24 h on an incubator shaker to disperse the oil throughout the broth in the tubes. From the tubes showing no bacterial growth, 0.1 mL of the cells was spread on triplettase soy agar plates in triplicate.

**In Vivo Study**

The experiment was performed at Centre de Formation Professionnelle Agricole dans le secteur de l’Aviculture (Sidi-Thabet, Tunisia). Seven hundred and fifty 1-d-old male broiler chickens (Arbor Acres, Société Tunisienne d’Aviculture, Borj-Cedria, Tunisia) were weighed, placed in 30 floor pens (i.e., 25 birds per pen; size: 2.4 × 1.3 m), and randomly assigned to 6 dietary treatment groups (5 replicates per treatment). Used litter, top-dressed with 7 cm of wood shavings, was utilized as bedding. The room temperature was gradually decreased from 32°C at d 1 to 24°C at d 22. The light was continuous during the first 3 d; then the lighting regimen was 23 h per day. One basal diet based on corn and soybean meal was formulated (Table 1) according to the nutritional requirements for chickens (Larbier and Leclercq, 1992) and calculated using a software (version 2.0, PORFAL, ITP-INRA, Paris, France). The basal diet was fed in mash form and contained no antibiotics or other growth factors (control; CON). The basal diet was supplemented with antibiotic (Avilamycin Maxus, Elanco Animal Health, Madrid, Spain) as antibiotic growth promoters (A) at the rate of 44 mg/kg of diet. To have the same amount of plant essential oils in the diet, the essential oil preparations were added to the basal diet at 100 mg/kg for rosemary (ROS) and oregano (OR), 50 mg of rosemary + 50 mg of oregano/kg (RO), or 1,000 mg/kg of BEO (essential oil mixture, EOM). The 6 diets were manufactured at a commercial company (Aliments Composés du Nord, Soliman, Tunisia). The diets were given to broiler chickens from 1 to 42 d of age. Feed and water were supplied ad libitum throughout the entire experiment. Fasted birds were weighed individually at 1, 21, and 42 d of age to determine BW. Feed intake was recorded by pen during the entire experiment.

**Statistical Analysis**

Data (antimicrobial activities and growth performances) were statistically analyzed for treatment effect by the ANOVA procedures of Statview software (SAS Inst. Inc., Cary, NC). The experimental unit was the floor pen, and the mean differences were determined using Fisher’s test of LSD. The level of statistical significance was set at \( P \leq 0.05 \).

**RESULTS**

**Chemical Composition**

The essential oils isolated from rosemary and oregano were characterized by their increased content of 1,8-cineole (49.99%) and carvacrol (69.55%), respectively (Table 2). The monoterpenes, which represented approximately 75% of the mixture, were the major chemical class of the rosemary essential oil. They were dominated by 1,8-cineole, the 5 other main components in this chemical class being α-pinene, β-pinene, camphene, cymol, and β-myrcene. Ketones were the second major class of compounds in rosemary essential oil with camphor (11.88%) being the only compound detected. In the group of alcohols, borneol and p-menth-1-en-8-ol
Table 1. Formulation and calculated composition of the broiler chicken diet (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, g/kg</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>628</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>332</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>12.7</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>18.8</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>1.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>2.5</td>
</tr>
<tr>
<td>Mineral and vitamin mixtures¹</td>
<td>4.5</td>
</tr>
<tr>
<td>Nutrient composition²</td>
<td></td>
</tr>
<tr>
<td>ME, MJ/kg</td>
<td>12.17</td>
</tr>
<tr>
<td>CP, g/kg</td>
<td>265</td>
</tr>
<tr>
<td>Lys, g/kg</td>
<td>11.5</td>
</tr>
<tr>
<td>Met, g/kg</td>
<td>4.8</td>
</tr>
<tr>
<td>Met + Cys, g/kg</td>
<td>8.4</td>
</tr>
<tr>
<td>Trp, g/kg</td>
<td>2.4</td>
</tr>
<tr>
<td>Ca, g/kg</td>
<td>10.8</td>
</tr>
<tr>
<td>Available P, g/kg</td>
<td>4.2</td>
</tr>
</tbody>
</table>

¹Mineral and vitamin mixtures supplied per kilogram of diet: Cu, 8.7 mg as copper sulfate pentahydrate; I, 1.2 mg as potassium iodate; Se, 0.2 mg as sodium selenite; Zn, 84 mg as zinc oxide; Fe, 44 mg as iron carbonate; Mn, 106 mg as manganese sulfate; vitamin A, 10,000 IU; cholecalciferol, 1,500 IU; vitamin E, 15 mg; butylated hydroxytoluene, 125 mg; menadione, 5 mg; thiamine, 0.5 mg; riboflavin, 4 mg; calcium pantothenate, 8 mg; niacin, 25 mg; pyridoxine, 1 mg; vitamin B12, 0.008 mg; folic acid, 1 mg; biotin, 0.2 mg; and choline chloride, 750 mg.

²Calculated using PORFAL software version 2.0 (ITP-INRA, Paris, France).

are the most abundant. Caryophyllene is the main component in the sesquiterpenes group, which represented more than 4% of the whole essential oil of rosemary. Finally, there are no phenols in the rosemary essential oil.

In the essential oil of oregano, the phenols (73.64%) represented the main chemical class. They were dominated by carvacrol (69.55%). The monoterpenes, which represented more than 18% of the whole oregano essential oils, were mainly represented by cymol, δ-terpinene, limonene, and α-pinene. Caryophyllene and α-bisabolene were the only 2 components of the sesquiterpenes group, which represented more than 4% of the whole essential oil of oregano. Alcohols were the minor chemical group in the oregano essential oil, and they were only represented by linalool and 1-terpinen-4-ol. The BEO were mainly represented by the aldehydes (cinnamaldehyde) and the monoterpenes (1,8-cineole) chemical groups.

Antimicrobial Activity of Essential Oils

The results of the present study showed that the essential oils had varying degrees of growth inhibition against the microorganisms tested. The disc diameters of the inhibition zones, the MIC and the MBC values of the rosemary, oregano, and BEO are shown in Tables 3, 4, and 5. The results of the disc diffusion method (Table 3) indicated that the rosemary essential oil had antibacterial activity against only 3 pathogenic bacteria, including E. coli, S. indiana, and L. innocua among the 5 tested bacteria. The E. coli, S. indiana, L. innocua, S. aureus, and B. subtilis were more sensitive to the oregano essential oil than the rosemary essential oil. The combination of the rosemary and oregano essential oils (vol/vol) did not exhibit greater antibacterial activity than the separate use of the rosemary or oregano essential oils. Thus, there was no synergy between compounds of rosemary and oregano essential oils except for B. subtilis. The essential oils of rosemary and oregano used alone or in combination were not efficient against L. plantarum, L. rhamnosus, L. reuteri, L. salivarius, E. faecium, and E. fecalis. The BEO showed an antimicrobial activity against all studied bacteria.

Table 2. Composition of essential oils of rosemary and oregano, % peak area

<table>
<thead>
<tr>
<th>Compound¹</th>
<th>Ki²</th>
<th>Rosemary</th>
<th>Oregano</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoterpenes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Pinene</td>
<td>930</td>
<td>9.95</td>
<td>1.37</td>
</tr>
<tr>
<td>Camphene</td>
<td>941</td>
<td>3.66</td>
<td>ND³</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>960</td>
<td>6.53</td>
<td>ND</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>974</td>
<td>1.08</td>
<td>0.62</td>
</tr>
<tr>
<td>α-Phellandrene</td>
<td>1,011</td>
<td>ND</td>
<td>0.10</td>
</tr>
<tr>
<td>α-Terpipene</td>
<td>1,018</td>
<td>0.40</td>
<td>ND</td>
</tr>
<tr>
<td>Cymol</td>
<td>1,026</td>
<td>1.60</td>
<td>10.57</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1,147</td>
<td>49.99</td>
<td>ND</td>
</tr>
<tr>
<td>δ-Terpipene</td>
<td>1,192</td>
<td>0.75</td>
<td>3.05</td>
</tr>
<tr>
<td>Carene</td>
<td>1,239</td>
<td>0.40</td>
<td>0.89</td>
</tr>
<tr>
<td>Limonene</td>
<td>1,449</td>
<td>ND</td>
<td>1.64</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>74.36</td>
<td>18.24</td>
</tr>
<tr>
<td>Ketone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camphor</td>
<td>1,421</td>
<td>11.88</td>
<td>ND</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>1,251</td>
<td>0.83</td>
<td>1.08</td>
</tr>
<tr>
<td>Borneol</td>
<td>1,555</td>
<td>3.45</td>
<td>ND</td>
</tr>
<tr>
<td>1-Terpinen-4-ol</td>
<td>1,562</td>
<td>0.95</td>
<td>1.15</td>
</tr>
<tr>
<td>p-Menth-1-en-8-ol</td>
<td>1,567</td>
<td>2.23</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>7.46</td>
<td>2.23</td>
</tr>
<tr>
<td>Phenol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymol</td>
<td>2,080</td>
<td>ND</td>
<td>4.09</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>2,125</td>
<td>ND</td>
<td>69.55</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0</td>
<td>73.64</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copaene</td>
<td>1,585</td>
<td>0.22</td>
<td>ND</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>1,600</td>
<td>3.89</td>
<td>3.97</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>1,855</td>
<td>0.43</td>
<td>ND</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>1,904</td>
<td>0.26</td>
<td>ND</td>
</tr>
<tr>
<td>α-Bisabolene</td>
<td>2,192</td>
<td>ND</td>
<td>0.13</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4.80</td>
<td>4.10</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicyclo(3.1.0)hexane</td>
<td>860</td>
<td>0.33</td>
<td>0.22</td>
</tr>
<tr>
<td>1-Octen-3-ol</td>
<td>968</td>
<td>ND</td>
<td>0.11</td>
</tr>
<tr>
<td>Bicyclo(4.1.0)hept-3-ene</td>
<td>985</td>
<td>0.26</td>
<td>ND</td>
</tr>
<tr>
<td>Bicyclo(2.2.1)heptan-2-ol</td>
<td>1,572</td>
<td>0.91</td>
<td>ND</td>
</tr>
<tr>
<td>1,4.7-Cycloundecatetraene</td>
<td>2,162</td>
<td>ND</td>
<td>0.22</td>
</tr>
<tr>
<td>1,5-Heptadiene</td>
<td>2,188</td>
<td>ND</td>
<td>0.21</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1.50</td>
<td>0.76</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>98.97</td>
<td></td>
</tr>
</tbody>
</table>

¹Identification by gas chromatography coupled to mass spectrometry (GC-MS): National Institute of Standards and Technology (Gaithersburg, MD).

²Ki: Kovats index on HP5-MS capillary column (Agilent Technologies, Paris, France). It converts retention times into system-independent constants.

³ND: compounds are not detected.
The MIC value of rosemary essential oil against *E. coli* was 4.40 mg/mL (Table 4). The *L. innocua* and *S. indiana* were inhibited only when the essential oil of rosemary was used at the concentration of 8.8 mg/mL. It is observed also that the essential oil of oregano exhibited a decreased MIC value (0.9 mg/mL) against *E. coli*, *L. innocua*, and *S. indiana* compared with the rosemary essential oil. The greatest MIC value (2.25 mg/mL) for oregano essential oil was obtained in the presence of *B. subtilis*. The combination of the essential oils of rosemary and oregano exhibited the same MIC values (1.11 mg/mL) for the 5 pathogenic bacteria but greater than those obtained for the oregano essential oil when used alone. Among pathogenic bacteria, *S. indiana* and *S. aureus* were more sensitive (MIC: 1.15 mg/mL) to the BEO compared with *E. coli*, *B. subtilis*, and *L. innocua* (MIC: 2.3 mg/mL). The 5 pathogenic bacteria showed increased susceptibility to the oregano essential oil. Moreover, *S. aureus* and *B. subtilis* were resistant only to the rosemary essential oil.

In many instances, the MBC values (Table 5) of the essential oils studied were equivalent to the MIC values (bactericidal effect). The essential oil of rosemary inhibited only the *E. coli* completely, and the MBC value detected was equivalent to the MIC value (4.4 mg/mL). Our results also showed that *E. coli*, *S. indiana*, *L. innocua*, and *S. aureus* were inhibited by the oregano essential oil at the concentration of 1.12 mg/mL, whereas *B. subtilis* was inhibited at the concentration of 2.25 mg/mL. The MBC values obtained in the case of the combination of essential oils of rosemary and oregano ranged from 1.11 to 4.45 mg/mL for *S. indiana*, *L. innocua*, and *S. aureus*, and they were greater than those observed in the presence of the oregano essential oil. The BEO exhibited a bactericidal effect against the 5 pathogenic bacteria, and the MBC values were greater.

### Table 3. Antibacterial activities by the disc diffusion method of essential oils against selected bacterial strains

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Rosemary</th>
<th>Oregano</th>
<th>Rosemary + oregano, vol/vol</th>
<th>BEO</th>
<th>CON−</th>
<th>CON+</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>8 ± 0.6</td>
<td>27 ± 1.0</td>
<td>17 ± 1.0</td>
<td>8.6</td>
<td>NA</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>Salmonella indiana</em></td>
<td>11 ± 1.0</td>
<td>23 ± 0.6</td>
<td>21 ± 0.6</td>
<td>21</td>
<td>NA</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>Listeria innocua</em></td>
<td>9 ± 0.6</td>
<td>21 ± 0.6</td>
<td>16 ± 0.6</td>
<td>17</td>
<td>NA</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>NA</td>
<td>12 ± 1.0</td>
<td>16 ± 0.6</td>
<td>17</td>
<td>NA</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>NA</td>
<td>NA</td>
<td>14 ± 0.6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>NA</td>
<td>NA</td>
<td>13 ± 1.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>Enterococcus fecalis</em></td>
<td>NA</td>
<td>NA</td>
<td>15 ± 0.6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*a–d* Mean values within a row having different superscripts are significantly different by the least significant difference test (*P* < 0.05).

Values are means ± SD of 3 values per test.

Carthago, Sousse, Tunisia.

BEO: commercial blend of essential oils (Phytosynthèse, Riom, France).

CON−: negative control, ethanol.

CON+: positive control, antibiotic (Chloramphenicol, Biomérieux, France).

NA: no antimicrobial activity.

### Table 4. Antimicrobial activity expressed as the minimum inhibitory concentration (MIC) of essential oils against selected bacterial strains

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Rosemary</th>
<th>Oregano</th>
<th>Rosemary + oregano, vol/vol</th>
<th>BEO</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>4.4</td>
<td>0.9</td>
<td>1.11</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Salmonella indiana</em></td>
<td>8.8</td>
<td>0.9</td>
<td>1.11</td>
<td>1.15</td>
</tr>
<tr>
<td><em>Listeria innocua</em></td>
<td>8.8</td>
<td>0.9</td>
<td>1.11</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>NA</td>
<td>0.9</td>
<td>1.11</td>
<td>1.15</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>NA</td>
<td>2.25</td>
<td>1.11</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*MIC* was considered the least concentration of each essential oil showing a clear zone of inhibition.

2Carthago, Sousse, Tunisia.

3BEO: commercial blend of essential oils (Phytosynthèse, Riom, France).

4NA: no antimicrobial activity.
than those obtained in the presence of oregano essential oil. The MBC values ranged from 1.15 to 2.3 mg/mL for *S. indiana*, *S. aureus*, *E. coli*, and *B. subtilis*; however, *L. innocua* needed a concentration of 4.6 mg/mL of BEO essential oil to be destroyed completely.

### Effect of Essential Oils on Broiler Performance

The effects of essential oils on BW of broiler chickens are presented in Table 6. Mortality was low (<5%) and independent of treatments. From 1 to 21 d of age, broiler chickens fed the CON diet grew less (*P* ≤ 0.05) than those fed the A, ROS, OR, RO, or EOM diets. Moreover, during the same trial period (1 to 21 d of age), there was no synergy between the essential oils of rosemary and oregano. Nevertheless, the broiler chickens receiving the ROS diet had better BW gain (*P* ≤ 0.05) than the groups fed A or OR diets from 1 to 21 d of age. In addition, from 22 to 42 d of age, broiler chickens fed the A diet grew faster (*P* ≤ 0.05) than those fed the CON diet. As observed during the first 3 wk of the trial, the decreased BW and BW gain obtained in the CON group from 22 to 42 d of age was completely alleviated (*P* ≤ 0.05) by rosemary, oregano, and BEO addition. The last 3 wk of the experiment, no differences in BW or BW gain were observed when animals were fed the A, ROS, OR, RO, or EOM diets. Throughout the entire experimental period, broiler chickens fed the CON diet had the least BW and BW gain (*P* ≤ 0.05). The supplementation of the basal diet with antibiotics or essential oils improved (*P* ≤ 0.05) broiler chicken BW and BW gain compared with the CON diet during the entire trial period. There were no differences in BW at 42 d of age or BW gain from 1 to 42 d of age when broiler chickens were fed A, ROS, OR, RO, or EOM diets.

Furthermore, broiler chickens receiving the A diet ate less feed (*P* ≤ 0.05) compared with the other groups fed CON, ROS, OR, RO, or EOM diets during the entire trial period. The greatest feed intake (*P* ≤ 0.05) was recorded in the RO group (3,892 g). The addition of oregano (3,771 g) and BEO (3,803 g) essential oils decreased (*P* ≤ 0.05) the feed intake of broiler chickens compared with the CON group (3,833 g). However, the ROS and CON groups had similar feed intake.

During the first 3 wk of the experimental period (1 to 21 d), there were no differences in feed efficiency among broiler chickens fed the CON diet and the ROS, OR, RO, or EOM diet. Moreover, the results of the present study showed that A, ROS, OR, RO, or EOM diets increased (*P* ≤ 0.05) feed efficiency compared with the CON diet from 22 to 42 d of age. In addition, throughout the entire experimental period (1 to 42 d of age), we observed the same feed efficiency as during the second growing period (22 to 42 d of age). In fact, the addition of antibiotic (group A) or essential oils (groups ROS, OR, RO and EOM) in the basal diet improved (*P* ≤ 0.05) the feed efficiency of broiler chickens compared with the CON group. Besides, there were no differences in feed efficiency when birds fed A, ROS, OR, RO, or EOM diets.

### DISCUSSION

#### Chemical Composition

The present study showed that the essential oil isolated from rosemary was characterized by its increased content of 1,8-cineole (49.99%). This result is close to work describing different essential oils of rosemary, in which the major component was 1,8-cineole and constituted 50.7% of the essential oil (Celiktas et al., 2007). Pintore et al. (2002) also reported that the major constituent of rosemary essential oils, collected from Morocco, Tunisia, Turkey, Greece, Yugoslavia, Italy, and France, was 1,8-cineole, which accounted for more than 40% of the essential oil. Moreover, Daferera et al. (2000) showed that the chemical composition of rosemary essential oil was also characterized by the predominant presence of 1,8-cineole, which accounted for 88.9% of the total essential oil. The rosemary essential oil studied in this study contained also camphor, α-pinene, β-pinene, caryophyllene, camphene, and borneol as mi-

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**Table 5.** Antimicrobial activity expressed as minimal bactericidal concentration (MBC) of essential oils against selected bacterial strains

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Rosemary²</th>
<th>Oregano²</th>
<th>Rosemary + oregano, vol/vol</th>
<th>BEO³</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>4.4</td>
<td>1.12</td>
<td>1.11</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Salmonella indiana</em></td>
<td>NA⁴</td>
<td>1.12</td>
<td>4.45</td>
<td>1.15</td>
</tr>
<tr>
<td><em>Listeria innocua</em></td>
<td>NA</td>
<td>1.12</td>
<td>2.22</td>
<td>4.6</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>NA</td>
<td>1.12</td>
<td>2.22</td>
<td>1.15</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>NA</td>
<td>2.25</td>
<td>1.11</td>
<td>2.3</td>
</tr>
</tbody>
</table>

¹The MBC was considered as the greatest dilution (least concentration) at which no growth occurred on the plates.

²Carthago, Sousse, Tunisia.

³BEO: commercial blend of essential oils (Phytosynthèse, Riom, France).

⁴NA: no antimicrobial activity.
Table 6. Effect of the essential oils on BW, BW gain, feed intake, and G:F in broiler chickens

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control²</th>
<th>Antibiotic³</th>
<th>Rosemary⁴</th>
<th>Oregano⁵</th>
<th>Rosemary + oregano⁶</th>
<th>EOM⁷</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0, g</td>
<td>47 ± 5</td>
<td>47 ± 5</td>
<td>48 ± 4</td>
<td>48 ± 5</td>
<td>48 ± 5</td>
<td>48 ± 5</td>
<td>0.68</td>
</tr>
<tr>
<td>d 21, g</td>
<td>597 ± 58c</td>
<td>615 ± 56b</td>
<td>633 ± 65c</td>
<td>619 ± 52b</td>
<td>627 ± 58b</td>
<td>627 ± 53b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>d 42, g</td>
<td>2,066 ± 204b</td>
<td>2,140 ± 226a</td>
<td>2,167 ± 243c</td>
<td>2,144 ± 232c</td>
<td>2,174 ± 257c</td>
<td>2,143 ± 224c</td>
<td>0.01</td>
</tr>
<tr>
<td>BW gain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0 to 21, g</td>
<td>551 ± 58c</td>
<td>566 ± 53b</td>
<td>585 ± 65c</td>
<td>569 ± 53b</td>
<td>580 ± 58ab</td>
<td>579 ± 52ab</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>d 21 to 42, g</td>
<td>1,473 ± 230b</td>
<td>1,540 ± 228b</td>
<td>1,521 ± 240b</td>
<td>1,525 ± 236b</td>
<td>1,565 ± 263b</td>
<td>1,512 ± 238b</td>
<td>0.04</td>
</tr>
<tr>
<td>d 0 to 42, g</td>
<td>2,025 ± 215c</td>
<td>2,093 ± 230b</td>
<td>2,119 ± 243c</td>
<td>2,096 ± 232c</td>
<td>2,133 ± 265b</td>
<td>2,095 ± 223b</td>
<td>0.02</td>
</tr>
<tr>
<td>Feed intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0 to 21, g</td>
<td>807 ± 15d</td>
<td>802 ± 11c</td>
<td>819 ± 11c</td>
<td>803 ± 10c</td>
<td>816 ± 16b</td>
<td>812 ± 16b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>d 21 to 42, g</td>
<td>3,028 ± 115c</td>
<td>2,907 ± 45c</td>
<td>3,008 ± 47c</td>
<td>2,968 ± 53d</td>
<td>3,075 ± 103c</td>
<td>2,995 ± 110c</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>d 0 to 42, g</td>
<td>3,833 ± 126c</td>
<td>3,709 ± 55c</td>
<td>3,827 ± 54c</td>
<td>3,771 ± 46d</td>
<td>3,892 ± 110c</td>
<td>3,803 ± 122c</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G:F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0 to 21</td>
<td>0.701 ± 0.079</td>
<td>0.689 ± 0.061</td>
<td>0.698 ± 0.080</td>
<td>0.681 ± 0.081</td>
<td>0.690 ± 0.085</td>
<td>0.694 ± 0.056</td>
<td>0.48</td>
</tr>
<tr>
<td>d 21 to 42</td>
<td>0.476 ± 0.032b</td>
<td>0.521 ± 0.017a</td>
<td>0.502 ± 0.036a</td>
<td>0.513 ± 0.032a</td>
<td>0.503 ± 0.088a</td>
<td>0.503 ± 0.029a</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>d 0 to 42</td>
<td>0.526 ± 0.018b</td>
<td>0.558 ± 0.012a</td>
<td>0.549 ± 0.017a</td>
<td>0.548 ± 0.012a</td>
<td>0.545 ± 0.012a</td>
<td>0.549 ± 0.013a</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Mean values within a row having different superscripts are different (P < 0.05).

1Values are means ± SD of 5 floor pens per dietary group.

2Without antibiotic or other growth factors.

3Included antibiotic (Avilamycin Maxus; Elanco Animal Health, Madrid, Spain) at the rate of 44 mg/kg.

4Rosemary (Carthago, Sousse, Tunisia) at the rate of 100 mg/kg.

5Oregano (Carthago, Sousse, Tunisia) at the rate of 100 mg/kg.

6Rosemary (50 mg/kg) and oregano (50 mg/kg).

7EOM: control + commercial blend of essential oils (Phytosynthèse, Riom, France) at the rate of 1,000 mg/kg.
nor components, whereas no verbenone was detected. 
Celiktas et al. (2007) showed that the essential oils of 
rosemary collected from İzmir in Turkey contained 
not only camphor (10.2%), α-pinene (11.5%), β-pinene 
(1.1%), Caryophyllene (0.6%), camphene (2.6%), and 
borneol (5.3%), but also verbenone (11.8%). 

The oregano essential oil was the second product 
studied in the present study; it contained carvacrol (69.55%) 
as the major constituent of the total oil. This 
carvacrol content is greater than those reported by Oussalahe et al. (2006), who studied 3 oregano varieties: the 
first (Origanum heracleoticum) from France, rich in car-
vacrol (54%); the second (Origanum compactum) from 
Morocco, rich both in carvacrol (22%) and γ-terpinene 
(23%); and the third (Origanum majorana) from Egypt, 
rich in terpinene-4-ol (26%) and γ-terpinene (12%). 
The reasons for this variability, in the chemical compo-
sition of the rosemary and oregano essential oils, can be 
due to the different geographical sources, the climate, 
the harvesting seasons, the genotype, the drying pro-
cedure, the distilled part of the plant (e.g., seeds, leaf, 
root, or bark), and the technique for processing (e.g., 
cold extraction, steam distillation, or extraction with 
nonaqueous solvents). All of these factors influence the 
relative concentration of each constituent in the essen-
tial oils (McGimpsey and Douglas, 1994; Venskutonis, 
1996). Finally it seems that essential oils of rosemary and 
oregano analyzed in this work could be classified as 
1,8-cineole type and carvacrol type, respectively. The 
BEO contained mainly aldehydes (cinnamaldehyde) 
and monoterpenes (1,8-cineole) as chemical groups. 
The presence of these components and others gives an-
timicrobial agent properties to rosemary, oregano, and 
BEO.

**Antimicrobial Activity of Essential Oils**

Our results showed that the essential oils of rosemary 
and oregano had antimicrobial activity against only 
the pathogenic bacteria, whereas the BEO was efficient 
against all the 11 studied bacteria (pathogenic and lactic 
acid bacteria). In fact, the essential oil of rosemary 
inhibited the growth of 3 pathogenic bacteria (E. coli, 
S. indiana, and L. innocua), but it destroyed only E. coli. 
Similar findings were obtained by Celiktas et al. (2007), who reported that E. coli was sensitive to the 
essential oil of rosemary. The antimicrobial activities of 
the rosemary essential oil can be attributed, in part, to 
the presence of increased concentrations of 1,8-cineole 
(Knobloch et al., 1989). Nevertheless, Lopes-Lutz et al. 
(2008) demonstrated that potent antimicrobial activities 
of the rosemary essential oils were not only correlated 
to the presence of 1,8-cineole. Camphor and borneol 
were previously reported to have potent antimicrobial 
activities in vitro against pathogenic bacteria (Kordali et al., 2005). Moreover, other minor constituents have 
also been reported for their antimicrobial activity such 
as α-pinene, β-pinene, limonene, α-terpinene, Caryophyl-
ylene, and camphene (Sökmen et al., 2004). Recently, 
Randrianariveloo et al. (2009) reported that E. coli was 
more sensitive to pure linalool than pure 1,8-cineole. 
Moreover, Celiktas et al. (2007) observed that rosemary 
essential oil containing verbenone (45.2%) exhibited the 
greatest antimicrobial activities compared with other 
rosemary essential oils characterized by a lack or low 
content of verbenone. Therefore, the weak antimicro-
bial activities of rosemary essential oils against patho-
genic bacteria tested in the present study were likely 
due to the lack of verbenone. In addition, we observed in 
our study that there were no antimicrobial activities 
of the rosemary essential oil against nonpathogenic 
bacteria (4 strains of Lactobacillus and 2 strains of Entro-
coccus). Our results were not consistent with those 
reported by Celiktas et al. (2007), who reported that 
essential oils of rosemary, collected from Canakkale in 
Turkey, which contained a greater proportion of verbe-
none (45.2%), had a great antibacterial activity against 
E. fæcals. Therefore, the antibacterial activity potential of 
rosemary essential oil against pathogenic and non-
pathogenic bacteria was related to the rate of verben-
one, camphor, and linalool rather than to the increased 
content of 1,8-cineole.

In the present study, the oregano essential oil showed 
interesting antimicrobial effects against the 5 studied 
pathogenic bacteria. These results are similar to those 
obtained by Oussalah et al. (2006) who studied 3 spe-
cies of oregano (Origanum heracleoticum, Origanum 
compactum, and Origanum majorana). Their studies 
showed that O. heracleoticum and O. compactum had 
the greatest antimicrobial activities against 4 patho-
genic bacteria (E. coli, Listeria monocytogenes, Salmo-
nella typhimurium, and S. aureus) because they had a 
greater concentration of carvacrol. Thus, the anti-
microbial activities of the oregano essential oil can be 
attributed to the presence of increased concentration 
of carvacrol (Dorman and Deans, 2000; Chorianopoulos 
et al., 2004). Thymol had also antimicrobial effects 
on the number of coliforms as reported by Michiels et 
al. (2009). In fact, carvacrol and thymol are isomeric 
chemicals with a phenolic functional moiety, and they 
had comparable antimicrobial properties (Michiels et 
al., 2009). Like rosemary, the nonpathogenic bacteria 
were not sensitive to the oregano essential oil in the 
present study. This is in disagreement with the results 
obtained by Michiels et al. (2009), who demonstrated 
that carvacrol had antimicrobial effects against lacto-
bacilli. This could be explained by differences in the 
sensitivity of lactobacilli bacteria species and strain 
tested in our study and that of Michiels et al. (2009). 
Second, we used a crude oregano essential oil that con-
tained many components in the present experiment, but 
Michiels et al. (2009) studied the antimicrobial effects 
of pure carvacrol. It has been reported in the literature 
that essential oil samples, studied as complex mixtures, 
may exhibit antimicrobial activities which differ from 
those of their major component studied alone (Delaquins 
et al., 2002). In fact, major or trace compounds con-
tained in an essential oil might increase or decrease its
antimicrobial activity because it should be taken into consideration the possible synergistic and antagonistic effect of compounds in the oil (Lopes-Lutz et al., 2008). Third, the methods used to evaluate the antimicrobial activities of essential oil were different in those studies. In our work, we used the disc diffusion method, but Michiels et al. (2009) used an in vitro incubation model, simulating the fermentation in the animal gastrointestinal tract.

However, we observed in the present study that the antimicrobial activities of oregano essential oils were greater than those of rosemary essential oil. In addition, there were no synergistic effects between essential oils of rosemary and oregano, except for *B. subtilis*. Ueltue et al. (2002) showed that *p*-cymene probably acts synergistically with carvacrol, and Savelev et al. (2003) found a minor synergy in 1,8-cineole/α-pinene and an antagonistic effect in 1,8-cineole/camphor combinations. However, to our knowledge, there are no data in the literature about the combination of rosemary and oregano essential oil compounds. Also, the present study indicates that the combination of oregano and rosemary essential oils (vol/vol) exhibited antibacterial activities only against studied pathogenic bacteria. This strongly indicates selective antimicrobial effects of rosemary and oregano essential oils used alone or in combination on pathogenic bacteria vs. nonpathogenic bacteria. Unfortunately, there is a little information on the effect of rosemary and oregano essential oils in the same study on both pathogenic and nonpathogenic bacteria growth to establish clear and consistent results about their selective properties. On the basis of their antimicrobial effects on gram-positive vs. gram-negative bacteria, the oregano and rosemary essential oils can be considered as nonselective because they inhibited *E. coli* and *S. indiana*, which are gram-negative, and *L. innocua* and *S. aureus*, which are gram-positive. This is in agreement with the results obtained by Burt (2004) and Michiels et al. (2009).

The BEO essential oil evaluated in the present study exhibited potent antimicrobial activities against all studied bacteria (i.e., pathogenic or nonpathogenic and gram-positive or gram-negative bacteria). Therefore, it can be considered as nonselective for both criteria. This might be attributed to the presence of aldehydes (cinnamaldehyde) in the BEO, which is in line with the results of Lee and Ahn (1998), Oussalah et al. (2006), and Michiels et al. (2009). In the current study, it seems that the ranking of antimicrobial activities against studied bacteria was cinnamaldehyde > carvacrol > 1,8-cineole. This is consistent with the results of Michiels et al. (2009), who found the following order: cinnamaldehyde > carvacrol > thymol > eugenol, but they did not test 1,8-cineole in their study. Nevertheless, the results of Friedman et al. (2002) showed the following rank: carvacrol > cinnamaldehyde > thymol > eugenol.

Globally, the antimicrobial mode of action of the BEO and the 2 essential oils tested in the present study is considered to arise mainly from their hydrophobic potential to introduce into the bacterial cell membrane, disintegrate membrane structures, and cause ion leakage (Windisch et al., 2008). Moreover, it is likely that essential oil components can penetrate into the interior of the cell and interact with intracellular sites critical for bacterial activities (Cristani et al., 2007). More precisely, they are able to inhibit glucosyltransferase enzyme activity, which is responsible in bacteria adhesion to its sites (Tsai et al., 2007). In summary, the essential oil preparations (rosemary, oregano, and BEO) tested in the present study had different chemical composition and different antimicrobial activities. Thus, we expect that they have different effects on broiler chicken performances.

**Effect of Essential Oils on Broiler Performances**

The results of the present study show that the suppression of antibiotic growth promoter markedly depressed growth performance of broiler chickens. Emborg et al. (2001) obtained similar results when broiler chickens were fed a diet without antibiotic growth promoter. The exact mode of action of the antibiotic growth promoter is not well established, and different mechanisms have been elaborated. Most are based on a reduction in bacterial numbers with a reduction in the production of growth-depressing microbial metabolites and in the competition for nutrients with the host (Anderson et al., 1999). Moreover, the selection of healthier microbial groups could constitute another mechanism of action (Castillo et al., 2006). The addition of rosemary, oregano, and BEO to a corn-based diet in the present study improved broiler chicken performance, but there were no interactions between rosemary and oregano essential oils as reported by Basmacioglu et al. (2004). This beneficial effect of essential oils has previously been reported in numerous studies (Hertampf, 2001; Alçiçek et al., 2003). The improvements of broiler chicken growth performance could be partly explained by the increase in the apparent digestibility of dietary protein and the prececal digestive capacity in general, which increase the intestinal availability of nutrients for absorption and consequently lead animals to grow faster (Windisch et al., 2008). Another mode of action of growth-promoting feed essential oils arises from stabilizing the ecosystem of the gastrointestinal microbiota (Windisch et al., 2008) by decreasing microbial activity and controlling potential pathogenic microorganisms in the gastrointestinal tract of animals (Castillo et al., 2006). In fact, animals receiving feed supplemented with essential oils had more stabilized intestinal health and are less exposed to microbial tox-
zymes (Jamroz et al., 2003). In addition, Jamroz et al. (2006) demonstrated that essential oil feed additives improved the absorptive capacity of the intestinal mucosa by increasing intestinal villi size and crypt depth. The depression of intraepithelial lymphocyte number in the jejunum of animals treated with essential oils proved that there is a relationship between the immune system and essential oils (Nofrarías et al., 2006).

All these findings might explain the beneficial effect of essential oils; however, some studies indicate that these feed additives are not efficient in broiler chickens (Lee et al., 2003; Botsoglou et al., 2004). In fact, Basmacioglu et al. (2004) and Botsoglou et al. (2005) showed that the use of rosemary and oregano essential oils alone or in combination did not improve laying hen performance or broiler chicken feed conversion. The large variation in the effects of essential oils on broiler chicken performance is due to intrinsic and extrinsic factors such as physiological status of animals, rearing environment, infections, diet composition, the content of active substances in essential oil sample, and the different experimental approaches used by the authors to test the suitability of these substances as growth-promoting feed additives for broiler chickens (Giannenas et al., 2003; Windisch et al., 2008).

Although the 3 essential oil preparations tested in the present study had different antimicrobial activities, they exhibited the same efficiency in broiler chickens. Thus, there was no relationship between in vitro essential oil antimicrobial potential and their efficiency in broiler chickens. To our knowledge, this finding has not previously been demonstrated. One possible hypothesis to explain this result is the decreased doses used in the in vivo trial compared with those used in the in vitro experiment. This may indicate that the antimicrobial potential of the essential oils should not be the same in in vivo and in vitro studies. Moreover, the essential oil antimicrobial activity tests were performed on pure bacteria and not on a mixture of microorganisms as found in the gastrointestinal tract of broiler chickens. In these circumstances, it seems that the effects of essential oils would be related to changes in the ecological structure and metabolic activity of the microbial community rather than to a reduction in the number of some bacteria groups (Castillo et al., 2006). It is likely that rosemary, oregano, and BEO used in smaller amounts had similar and beneficial effects on the ecosystem of broiler chicken gastrointestinal microbiota. A second plausible hypothesis is that the beneficial effects of the essential oils should not only arise from their antimicrobial properties, but also from their interference with digestive and absorptive processes and the immune system (Windisch et al., 2008).

In summary, the suppression of antibiotic growth promoters from feed decreased zootechnical performance of broiler chickens. The addition of rosemary, oregano, and BEO counteracted these effects. These 3 essential oil preparations had the same efficiency in broiler chickens despite their different antimicrobial activities.

Thus, the selection of the essential oils as effective feed additives on the basis of their in vitro antimicrobial activities is not sufficient and should be completed by in vivo experiments. More specific studies are required to clarify how essential oils affect the gastrointestinal microbiota of broiler chickens for better use under field conditions.

LITERATURE CITED


