Broiler production leads to the accumulation of various pollutants in poultry houses, including bacteria and their metabolites: endotoxins, toxic gases, and aroma compounds (Seedorf et al., 1998; Baykov and Stoyanov, 1999; Bakutis et al., 2004; Tymczyna et al., 2007; Vučemilo et al., 2007; Schulz et al., 2011). Pollution levels increase steadily, and sick building syndrome is often reported at the end of the production process. Microbiological contamination is also observed, in particular a steep increase in the levels of aerobic mesophilic bacteria, the predominant group of pathogenic microbes. The number of bacteria released into the environment increases as chickens age, and pathogenic microbes are found as far as 3 km from poultry houses (Baykov and Stoyanov, 1999).

In addition to gram-positive cocci (Staphylococcus, Streptococcus, Micrococcus) and bacilli (Bacillus), poultry houses are also colonized by gram-negative bacteria of the family Enterobacteriaceae, including Escherichia coli, Salmonella spp., Shigella spp., and Klebsiella spp., as well as Pseudomonas spp., Acinetobacter spp., and Flavobacterium spp. (Davis and Morishita, 2005; Tymczyna et al., 2007; Vučemilo et al., 2007; Brödka et al., 2012). The cell membranes of pathogenic gram-negative bacteria contain lipopolysaccharide complexes. Those endotoxins are released when bacterial cells disintegrate, and they are present in high concentrations in poultry houses (Baykov and Stoyanov, 1999).

The antimicrobial properties of essential oils have been demonstrated by various in vitro studies, whereas their effect on poultry farm hygiene has not been thoroughly investigated, in particular with reference to aerial treatment. The present study aims to assess the antibacterial effects of natural essential oils in broiler houses. Two experimental rooms were fogged with aqueous solutions of peppermint and thyme oils. The control room was sprayed with pure water. The experiment was conducted on broilers aged 1 to 42 d. The rooms were fogged every 3 d. One day after fogging, the total counts of mesophilic aerobic bacteria, Enterobacteriaceae, and mannitol-positive staphylococci were determined. Samples were collected from the air, litter, walls, and drinkers. The results of the study demonstrate that essential oil mist may improve hygiene standards in broiler farms. During broiler growth, the mean total counts of mesophilic bacteria in the rooms treated with essential oils were lower (P < 0.01 or P < 0.05) in comparison with the control. Enterobacteriaceae and staphylococci counts were also higher in the control group. A single exception was noted in a litter sample where the mean count of Enterobacteriaceae in the room fogged with peppermint oil was higher than in the control. Both oils reduced bacterial counts, but thyme oil was more effective in reducing coliform bacteria, whereas peppermint oil had a higher inhibitory effect on the proliferation of staphylococci. These promising results encourage further research to determine the optimal doses and the effects of essential oils and their combinations on the living conditions and health status of broiler chickens.

Key words: essential oil, bacteria, broiler house, Enterobacteriaceae, Staphylococcus

2013 Poultry Science 92:2834–2843
http://dx.doi.org/10.3382/ps.2013-03147

© 2013 Poultry Science Association Inc.
Received February 28, 2013.
Accepted July 1, 2013.
1 Corresponding author: dorota.witkowska@uwm.edu.pl
of in vitro studies have demonstrated that essential oils eliminate pathogenic bacteria, among them E. coli, Salmonella spp. (including Salmonella Enteritidis and Salmonella Typhimurium), Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermis, Klebsiella pneumoniae, Shiella spp., Proteus vulgaris, and Bacillus cereus (Hammer et al., 1999; Inouye et al., 2003; Azaz et al., 2004; Bagamboula et al., 2004; Peñalver et al., 2005; Kędzia and Holderna-Kędzia, 2007).

Thyme essential oil (Oleum thymi) extracted from the common thyme (Thymus vulgaris L.) demonstrates high levels of antimicrobial activity, and it has been frequently compared with other oils in both in vitro (Hammer et al., 1999; Lutomski and Kędzia, 2000; Azaz et al., 2004; Bagamboula et al., 2004; Peñalver et al., 2005; Al-Bayati, 2008) and in vivo studies (Mitsch et al., 2004). Thyme plants contain around 0.5 to 2.5% oil, and thyme essential oil is made up of thymol (45–47%), p-cymene (32–34%), carvacrol (4–5%), γ-terpinene, linalool, and α-pinene (Bagamboula et al., 2004). The oil extracted from selected thyme varieties may contain up to 60% carvacrol (Azaz et al., 2004). Thymol and carvacrol (phenolic compounds) are thyme oil ingredients with the strongest antiseptic properties (Bagamboula et al., 2004).

Peppermint essential oil (Oleum menthae piperitae), obtained from peppermint plants (Mentha piperita), is also a highly potent antimicrobial substance whose composition differs from thyme oil. Peppermint oil contains mainly menthol (63%) and p-menthone (19.5%) as well as ketones, monoterpenes, menthofuran, and terpene oxides (Inouye et al., 2001; Kędzia, 2007).

Essential oils attracted the interest of European animal breeders after the use of antibiotic growth-promoters was banned in animal nutrition. In addition to herbs, spices, and plant extracts, essential oils offer a viable alternative to feed antibiotics due to the content of various active substances. Herbal feed additives are widely used, and some formulas include essential oils (Hernández et al., 2004; Mitsch et al., 2004). Commercially available products containing essential oils can be added to drinking water or used in the fumigation of poultry houses, but their effects on the living environment of birds have not been investigated in detail to date. The use of essential oil vapor could improve poultry house hygiene. Inouye et al. (2001, 2003) observed that essential oils in liquid and vapor form inhibited the growth of selected bacteria and fungi under laboratory conditions. The above authors found that essential oil vapors were much more effective because water vapor had an additional influence on microbial cells. They concluded that essential oils should be optimally used over short periods of time, but at high water vapor concentrations. Positive results of in vitro studies encourage further research into the effects of essential oils on the living environment of chickens.

The aim of this study was to evaluate the antibacterial effect of natural thyme and peppermint essential oils in broiler houses.

**MATERIALS AND METHODS**

**Chicken Housing and Experimental Design**

The experimental design and procedures were approved by the Local Ethics Committee for Animal Experimentation at the University of Warmia and Mazury in Olsztyn. The experiment was performed in spring on 360 unsexed Ross 308 broiler chickens aged 1 to 42 d. One-day-old chicks were divided into 3 groups (control group and 2 experimental groups) of 120 birds each. Broilers were housed in 3 separate, climate-controlled (forced-air ventilation), and identically equipped rooms with a floor area of 9.25 m² and a cubic capacity of 26.2 m³. Each experimental room was cleaned and disinfected before stocking. Chickens were kept on straw litter in accordance with standard requirements. Initial ambient temperature was set at 32°C, and it was gradually decreased to 20°C in wk 6. Each room was provided with ad libitum access to feed and water. Birds were fed commercial starter, grower, and finisher pellet diets (Rolpol, Uścikowo, Poland) containing wheat and soybean extracts and Schaumann premixes (Gniezno, Poland). Chicks were vaccinated in the hatchery against infectious bronchitis, infectious bursal, Marek’s, and Newcastle diseases.

Commercially available 100% natural essential oils (ETJA Corp., Elblag, Poland) were used in the experiment. The experimental broiler rooms were fogged with aqueous solutions of peppermint oil (Mentha piperita) and thyme oil (Thymus vulgaris L.). Hot water was mixed with essential oils in doses recommended by the manufacturer for human aerosol therapy (based on the cubic capacity of fogged rooms). The initial concentration of 1:500 was increased to 1:250 at 28 d before slaughter. The experimental rooms were fogged with 0.5 L of each oil solution before stocking and every third day during the rearing period. The control room was sprayed with 0.5 L of pure water. A fogger (Mgl-E, Poltech Corp., Warsaw, Poland) was used to produce aerosol particles smaller than 50 μm.

**Sampling and Microbiological Analysis**

Bacterial contamination of samples collected from the air, litter, walls, and drinkers was evaluated 1 d after fogging (every 3 d). Quantitative analyses of mesophilic aerobic bacteria, Enterobacteriaceae pathogens, and mannitol salt-positive staphylococci were carried out. Bacterial counts were also determined before stocking before and after essential oil treatments.

Air samples were collected with an air sampler (air IDEAL 3P, bioMérieux Corp., Craponne, France) at 5 locations in each room—in the center and at 2 points along 2 diagonals. The sampler, which operates on the Andersen impaction principle, has air intake of 100 L/min and impact speed of less than 20 m/s. Air volumes ranged between 5 and 100 L, subject to the type of medium and the expected contamination levels. Bacterial...
counts were determined in Petri dishes (90 mm) with solid media (Merck Corp., Darmstadt, Germany). Mesophilic bacteria were cultured on tryptic soy agar with casein-peptone and soy meal-peptone (TSA). The cultures were incubated at 35°C for 24 h. Enterobacteriaceae were cultured on crystal-violet neutral-red bile glucose agar according to Mossel et al. (1962; VRBD) for 24 h at 35°C. Red to dark purple colonies surrounded by a bright yellow zone were counted after 48 h of incubation at 35°C. The number of colonies on Petri dishes was determined using an automated colony counter (Schnett colonyQuant, Schnett-Biotec Corp., Göttingen, Germany). The results were corrected using Feller’s conversion formula, and bacterial counts were expressed in terms of cfu per meter$^3$ of air. The microbiological contamination of walls and drinkers was evaluated based on smear samples collected in accordance with standard PN-ISO 18593:2005 (2005). Two swab samples from the walls and drinkers in each room were collected in every analytical series. Sterile templates measuring 10 cm × 10 cm and sterile cotton swabs moistened with sterile peptone saline were used to obtain samples from flat wall surfaces. Swab samples from drinkers were collected from the same area each time. Swab samples were directly immersed in peptone saline solution (Merck Corp.) and shaken vigorously. Bulk samples of litter comprised 5 random samples collected with a sterile spatula. One gram of each sample was diluted 1:9 (wt/vol) in sterile peptone saline. All samples collected from the walls, drinkers, and litter were subjected to 10 sequential dilutions 1:9 (vol/vol). One milliliter of each dilution was inoculated in double Petri dishes with TSA, VRBD, and MSA media (Merck Corp.). The dishes were incubated at 35°C for 24 h (TSA and VRBD) or 48 h (MSA). Bacterial colonies were expressed in terms of cfu/100 cm$^2$ of wall surface, cfu per unit of drinker area, and cfu per 1 g of litter. Bacterial assays were performed according to Merck and PN-EN ISO 21528–2:2005 (2005) standards.

**Statistical Analysis**

Before statistical analysis, all bacterial counts were log$_{10}$ transformed to achieve normal distribution and calculate the mean, SD, minimum, and maximum values. The data present in Tables 4 to 6 were collected after fogging (every 3 d) and are reported as an average of the entire chicken rearing. The normality check for each group was performed by the Kolmogorov-Smirnov test. All data were normally distributed and were subjected to one-way ANOVA in Statistica 10 for Windows (StatSoft Inc., Tulsa, OK). The mean values of treatment groups were compared by Duncan’s multiple range test, and differences were considered at $P < 0.01$ and $P < 0.05$. Figures 1 to 3 show the trend lines of bacterial reductions by age and essential oil treatment, and the data points are the averages of 2 measurements from each rearing week.

**RESULTS**

Tables 1, 2, and 3 present the results of environmental monitoring of bacteria in control and experimental rooms before stocking. After the rooms had been prepared for rearing, the average counts of aerial mesophilic bacteria were similar in each room at around 2.6 log$_{10}$ cfu/m$^3$. The total number of mesophilic bacteria in litter was approximately 4.2 log$_{10}$ cfu/g in 2 rooms, whereas a difference of 0.3 log$_{10}$ was reported in the third room. The contamination of walls was determined at 1.0 log$_{10}$/100 cm$^2$, and somewhat higher contamination levels were noted in drinkers (1.3–1.7 log$_{10}$). The number of aerial mannitol-positive staphylococci was comparable with or lower than total mesophilic counts (in one of the rooms). Litter was considerably less contaminated with staphylococci than with mesophilic bacteria. On the surfaces of walls and drinkers, staphylococci were not identified or their counts were below 0.7 log$_{10}$. The presence of Enterobacteriaceae was not determined in disinfected rooms before stocking with a single exception in one room where minimal litter contamination (0.2 log$_{10}$/g) was observed. Three days later, 1 d after essential oil fogging, an increase was reported in mesophilic counts in the air and on control room surfaces (Table 1). In experimental rooms, the count of aerial mesophilic bacteria after essential oil treatment was identical to that noted after disinfection. The levels of contamination on the surfaces of walls and drinkers were lower (around 0.6–1.2 log$_{10}$) in comparison with the previous analysis. Mesophilic counts in litter were similar in each room (around 5.0 log$_{10}$/g) and approximately 1.0 log$_{10}$ higher than during previous determinations. A similar trend was observed with regard to staphylococci counts in litter, but in the room treated with peppermint oil, contamination levels were roughly 2.0 log$_{10}$ higher in comparison with the previous examination. The walls were colonized by staphylococci only in the control room (1.0 log$_{10}$), whereas drinkers were contaminated in the control room and in the room fogged with thyme oil (around 2.0 log$_{10}$). In those rooms, staphylococci counts were 2-fold higher (above 3.0 log$_{10}$/m$^3$) in comparison with the room where peppermint oil was used. Enterobacteriaceae were identified only in litter, and their population size ranged from 0.60 (control) to 1.36 (peppermint oil treatments) log$_{10}$/g.

The average total mesophilic counts in the air, litter, and on wall and drinker surfaces during broiler rearing are presented in Table 4. The mean aerial contamination of the analyzed rooms ranged from below 5.5 to above 5.8 log$_{10}$/m$^3$, and it was higher in the control room ($P = 0.002$) than in the rooms treated with essential oils. No statistically confirmed differences were observed between essential oil groups, but the lowest
Figure 1. Total mesophilic counts in the air (a), in litter (b), on walls (c), and on drinkers (d) from wk 1 to 6. Averages of 2 measurements each week.

Figure 2. Enterobacteriaceae counts in the air (a), in litter (b), on walls (c), and on drinkers (d) from wk 1 to 6. Averages of 2 measurements each week.
mean and maximum values were noted in the room treated with thyme oil. A similar trend was observed with regard to wall contamination, but the differences between control and experimental groups were determined at \( P = 0.025 \) and total mesophilic counts ranged from around 2.4 in rooms fogged with essential oils to 3.3 \( \log_{10} \)/100 cm\(^2\) in the control room. It should be noted that the maximum total bacterial concentrations on control room walls were approximately 5.2 \( \log_{10} \), whereas in rooms fogged with essential oils, the maximum values did not exceed 3.6 \( \log_{10} \) (thyme oil) and 3.8 \( \log_{10} \) (peppermint oil). Total bacterial counts on drinker surfaces in the room treated with thyme oil were lower \( (P = 0.045) \) than in the control room \( \) by 0.5 \( \log_{10} \). No statistically confirmed differences were noted between other groups, but the mean total mesophilic counts in the room fogged with peppermint oil were lower in comparison with the control room. The minimum and maximum values in the room treated with thyme oil were around 1 logarithm lower than in the other rooms. The average total count of litter bacteria was consistently highest in the control group at approximately 8.9 \( \log_{10} \)/g, and it was lowest in the thyme oil group at 8.2 \( \log_{10} \)/g \( (P = 0.031) \). The maximum and minimum total bacterial counts in litter revealed the same trend (Table 4). Figure 1 presents total mesophilic counts in the investigated rooms throughout the entire rearing period. The curve representing total aerial bacterial populations (Figure 1a) is similar for all groups, and bacterial counts in each week of the experiment ranged from 4.9 to 5.6 \( \log_{10} \)/m\(^3\) (essential oils) to 5.3 to 6.0 \( \log_{10} \)/m\(^3\) (control), excluding the second week when they increased to 6.0 (thyme oil), 6.2 (peppermint oil), and 6.4 \( \log_{10} \)/m\(^3\) (control). Total concentrations of aerial bacteria were highest in the control room and lowest in the room treated with thyme oil. A similar trend was observed on the surface of drinkers and in litter (Figure 1b and 1d), but total bacterial populations of walls (Figure 1c) in the second half of the rearing period were smaller in the room treated with peppermint oil. Total bacterial counts in litter and on drinker surfaces were characterized by a growing trend in the control room, whereas greater variations were reported in experimental rooms. A noticeable drop in the size of bacterial populations contaminating surfaces was observed in the third week of the study in the room treated with thyme oil (Figure 1c).

The mean *Enterobacteriaceae* counts in birds environment throughout the entire rearing period are shown in Table 5. The control room was characterized by the highest concentrations of aerial bacteria \( (1.8 \log_{10}; \) \( P = 0.041) \), as well as pathogens colonizing the walls \( (1.2 \log_{10}) \) and drinkers \( (2.5 \log_{10}) \). In the experimental room treated with thyme oil, *Enterobacteriaceae* counts on wall surfaces were lower \( (P = 0.005) \) than in the control room \( (1.0 \log_{10}) \). The differences \( (P = 0.037) \) were also observed between the above rooms in the populations of aerial bacteria and pathogens colonizing drinkers. *Enterobacteriaceae* counts determined in the

---

**Figure 3.** Mannitol salt-positive staphylococci counts in the air (a), in litter (b), on walls (c), and on drinkers (d) from wk 1 to 6. Averages of 2 measurements each week.
EFFECTIVENESS OF ESSENTIAL OIL MIST

Table 1. Total mesophilic counts (log$_{10}$ cfu) in the air, on walls, on drinkers, and in litter before stocking

<table>
<thead>
<tr>
<th>Place</th>
<th>Before essential oil treatment</th>
<th>After essential oil treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Peppermint oil</td>
</tr>
<tr>
<td>Air (m$^3$)</td>
<td>2.62</td>
<td>2.65</td>
</tr>
<tr>
<td>Walls (100 cm$^2$)</td>
<td>1.23</td>
<td>1.11</td>
</tr>
<tr>
<td>Drinkers (unsized)</td>
<td>1.70</td>
<td>1.45</td>
</tr>
<tr>
<td>Litter (g)</td>
<td>4.22</td>
<td>3.91</td>
</tr>
</tbody>
</table>

Table 2. Enterobacteriaceae counts (log$_{10}$ cfu) in the air, on walls, on drinkers, and in litter before stocking

<table>
<thead>
<tr>
<th>Place</th>
<th>Before essential oil treatment</th>
<th>After essential oil treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Peppermint oil</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Air (m$^3$)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Walls (100 cm$^2$)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Drinkers (unsized)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Litter (g)</td>
<td>0.00</td>
<td>0.20</td>
</tr>
</tbody>
</table>

DISCUSSION

This study demonstrated that essential oil fogging improves hygiene standards in broiler houses. The use of essential oils before stocking reduced bacterial counts in the air and on wall and drinker surfaces in experimental rooms and reduced the populations of mannitol-positive staphylococci in the room treated with peppermint oil solution. Oil treatment had no significant effect on litter hygiene (Tables 1 to 3). During broiler rearing, the mean total counts of mesophilic bacteria, Enterobacteriaceae, and staphylococci were also highest in the control group (Tables 4 to 6). A single exception was noted in litter where the average Enterobacteriaceae concentrations in the room treated with peppermint oil were higher than in the control. Bacterial contamination levels were reduced by both oils, but thyme oil demonstrated a stronger antibacterial effect with regard to coliform bacteria. Peppermint oil proved to be a more potent inhibitor of staphylococci (Table 6). Hammer et al. (1999) performed an in vitro study investigating the activity of 52 plant oils and extracts against various bacteria, including Salmonella Typhimurium, E. coli, Klebsiella pneumoniae, Serratia marcescens, and Staphylococcus aureus, and thyme oil was characterized by the lowest minimum inhibitory concentrations against E. coli. The results of the cited study demonstrate that thyme oil is a more effective inhibitor of the above bacteria, excluding Salmonella Typhimurium, than peppermint oil. Inouye et al. (2001,
2003) also observed that thyme oil is one of the most effective agents against respiratory tract pathogens by gaseous contact in laboratory conditions. In the above study, thyme oil had greater antibacterial activity against *E. coli*, *S. aureus*, and other bacteria than peppermint and other oils. The antimicrobial properties of thyme and other essential oils have been demonstrated by various in vitro studies (Friedman et al., 2002; Azaz et al., 2004; Bagamboula et al., 2004; Al-Bayati, 2008). Peñalver et al. (2005) analyzed the effect of essential oils on pathogenic intestinal *Enterobacteriaceae* strains of poultry origin. They reported the most satisfactory results in respect of oils containing carvacrol and thyme. Their findings justify further research into the use of essential oils for the prevention of diseases caused by *Salmonella*. In another study, the addition of essential oils to feed significantly lowered *Clostridium perfringens* counts in the intestines and feces of broilers (Mitsch et al., 2004), thus lowering the incidence of necrotic enteritis (Jerzsele et al., 2012). Diets supplemented with plant extracts and essential oils improved nutrient digestibility and performance results in broiler chickens (Hernández et al., 2004; Cross et al., 2007).

According to Mickienė et al. (2011), selected essential oils offer a promising alternative to other air disinfection methods in animal houses. The effect of essential oils on hygiene standards during broiler production has not been thoroughly investigated. Mituniewicz et al. (2008) applied Profistreu, a commercial preparation containing organic and inorganic active substances as well as essential oil to litter, and observed an average reduction of 0.33 log10 in total counts of aerial bacteria in broiler houses. Bakutis et al. (2011) investigated the effectiveness of pine and eucalyptus oils and their combinations in a hen house. A combination of the analyzed oils proved to be the most effective inhibitor of aerial bacteria in the above experiment. In our investigation, a decrease in total aerial bacterial counts was also noted, but our experiment differed with regard to the applied types and concentrations of essential oil, the form and period of their administration, the evaluated species, and environmental conditions. In a study by Vučemilo et al. (2007), microbial pollution levels in poultry houses varied significantly due to differences in housing standards and the applied research methods. In a 6-wk-long study of a commercial farm, Vučemilo et al. (2007) reported an increase in bacterial counts from 4.2 log10 cfu/m3 in the first week to 5.3 log10 cfu/m3 in the fifth week. In an experiment by Baykov and Stoyanov (1999), bacterial counts increased from 3.1 log10 cfu/m3 at the beginning of the production cycle to 5.2 log10 cfu/m3 on d 56. In our study, a noticeable increase in the counts of aerial bacteria was reported in the second week of broiler rearing (Figure 1). Similar results were reported by Wójcik et al. (2010) who observed differences in bacterial concentrations between winter and summer seasons.

In a study by Brooks et al. (2010), staphylococci accounted for at least 90% of cultured aerobic bacteria in broiler houses. According to Schulz et al. (2011), staphylococci are more useful indicators of bioaerosol emissions from broiler houses than total bacterial counts that may also represent other sources of pollution. In our study, aerial staphylococci were characterized by a similar proliferation pattern to that of total bacteria during broiler rearing (Figure 3a). The reduction in *Staphylococcus* populations in the rooms treated with essential oils was similar to the decrease in total cell counts.

Essential oils were most effective in eliminating *Enterobacteriaceae* colonizing wall surfaces whose populations were reduced by 80% in the room treated with thyme oil. Kašková et al. (2006) investigated the effectiveness of various sanitation methods in poultry houses to observe that mechanical cleaning decreased *Enterobacteriaceae* counts on wall surfaces by 86%. In general, surface cleaning and disinfection methods applied in the above experiment were more effective in eliminating pathogens than the essential oils investigated in our study.

Essential oils were the least potent inhibitors of microbial activity in litter. Pope and Cherry (2000) observed that PLT (Poultry Litter Treatment, composed of sodium bisulfate) eliminated litter bacteria, but the noted results were not statistically confirmed. They concluded that PLT has the potential to reduce *E. coli* and *Salmonella* populations in broiler house litter, but it is not capable of eliminating those pathogens entirely.

Our results indicate that essential oil fogging in poultry houses improves hygiene standards, but their efficacy seems to be lower than that of conventional disinfectants used by the authors mentioned previously in the Discussion. Therefore, there is a need to compare their effectiveness to the efficacy of disinfectants. The chemical composition of essential oils is difficult to standardize (Peñalver et al., 2005; Cross et al., 2007).

### Table 3. Mannitol salt-positive staphylococci counts (log10 cfu) in the air, on walls, on drinkers, and in litter before stocking

<table>
<thead>
<tr>
<th></th>
<th>Before essential oil treatment</th>
<th>After essential oil treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Peppermint oil</td>
</tr>
<tr>
<td><strong>Air (m³)</strong></td>
<td>2.03</td>
<td>2.28</td>
</tr>
<tr>
<td><strong>Walls (100 cm²)</strong></td>
<td>0.00</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Drinkers (unsized)</strong></td>
<td>0.73</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Litter (g)</strong></td>
<td>1.57</td>
<td>1.10</td>
</tr>
</tbody>
</table>
Table 4. Total mesophilic counts (log10 cfu) in the air, on walls, on drinkers, and in litter during broiler rearing

<table>
<thead>
<tr>
<th>Place</th>
<th>n</th>
<th>P-value</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air (m³)</td>
<td>60</td>
<td>0.002</td>
<td>5.81A</td>
<td>4.75</td>
<td>6.66</td>
<td>0.42</td>
<td>5.61B</td>
<td>4.56</td>
<td>6.44</td>
<td>0.49</td>
<td>5.46B</td>
<td>4.59</td>
<td>6.37</td>
<td>0.46</td>
</tr>
<tr>
<td>Walls (100 cm²)</td>
<td>24</td>
<td>0.025</td>
<td>3.27a</td>
<td>1.45</td>
<td>5.19</td>
<td>1.05</td>
<td>2.36b</td>
<td>0.60</td>
<td>3.80</td>
<td>0.98</td>
<td>2.43b</td>
<td>1.00</td>
<td>3.64</td>
<td>1.02</td>
</tr>
<tr>
<td>Drinkers (unsized)</td>
<td>24</td>
<td>0.045</td>
<td>4.81a</td>
<td>3.20</td>
<td>5.64</td>
<td>0.73</td>
<td>4.63ab</td>
<td>3.43</td>
<td>5.46</td>
<td>0.56</td>
<td>4.30b</td>
<td>2.22</td>
<td>4.93</td>
<td>0.79</td>
</tr>
<tr>
<td>Litter (g)</td>
<td>24</td>
<td>0.031</td>
<td>8.92a</td>
<td>7.25</td>
<td>9.86</td>
<td>0.68</td>
<td>8.45ab</td>
<td>7.18</td>
<td>9.52</td>
<td>0.70</td>
<td>8.20b</td>
<td>7.00</td>
<td>9.22</td>
<td>0.81</td>
</tr>
</tbody>
</table>

A,B Means within a row with different superscripts differ (P < 0.01).

a,b Means within a row with different superscripts differ (P < 0.05).

1Mean values are the averages of each measurement (every 3 d) from wk 1 to 6.

---

Table 5. Enterobacteriaceae counts (log10 cfu) in the air, on walls, on drinkers, and in litter during broiler rearing

<table>
<thead>
<tr>
<th>Place</th>
<th>n</th>
<th>P-value</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air (m³)</td>
<td>60</td>
<td>0.041</td>
<td>1.79a</td>
<td>0.00</td>
<td>3.16</td>
<td>0.78</td>
<td>1.61ab</td>
<td>0.00</td>
<td>2.96</td>
<td>0.90</td>
<td>1.47b</td>
<td>0.00</td>
<td>2.78</td>
<td>0.81</td>
</tr>
<tr>
<td>Walls (100 cm²)</td>
<td>24</td>
<td>0.005</td>
<td>1.21A</td>
<td>0.00</td>
<td>2.72</td>
<td>1.02</td>
<td>0.81AB</td>
<td>0.00</td>
<td>2.54</td>
<td>0.61</td>
<td>0.24b</td>
<td>0.00</td>
<td>1.04</td>
<td>0.21</td>
</tr>
<tr>
<td>Drinkers (unsized)</td>
<td>24</td>
<td>0.037</td>
<td>2.45a</td>
<td>1.00</td>
<td>3.45</td>
<td>0.69</td>
<td>2.34ab</td>
<td>0.85</td>
<td>3.92</td>
<td>0.90</td>
<td>1.67b</td>
<td>0.30</td>
<td>2.97</td>
<td>0.91</td>
</tr>
<tr>
<td>Litter (g)</td>
<td>24</td>
<td>0.488</td>
<td>6.28</td>
<td>4.48</td>
<td>8.15</td>
<td>1.08</td>
<td>6.42</td>
<td>5.00</td>
<td>8.08</td>
<td>1.06</td>
<td>6.09</td>
<td>4.48</td>
<td>8.08</td>
<td>1.22</td>
</tr>
</tbody>
</table>

A,B Means within a row with different superscripts differ (P < 0.01).

a,b Means within a row with different superscripts differ (P < 0.05).

1Mean values are the averages of each measurement (every 3 d) from wk 1 to 6.
The main advantage of essential oils is that they do not lead to the increase of microbial resistance, and unlike disinfectants and antibiotics, oil residues are not found in final products (Peñalver et al., 2005). The results of our study encourage further research to determine the optimal doses and effects of essential oils and their combinations on the living environment and health status of broiler chickens.

ACKNOWLEDGMENTS

The authors thank Edyta Mituniewicz and Jerzy Powązka (Faculty of Animal Bioengineering, University of Warmia and Mazury, Olsztyn, Poland) for their technical support.

REFERENCES


Inouye, S., S. Abe, H. Yamaguchi, and M. Asakura. 2003. Comparative study of antimicrobial and cytotoxic effects of selected es-


