The Microbe 6 (2025) 100248

Contents lists available at ScienceDirect

The Microbe

journal homepage: www.sciencedirect.com/journal/the-microbe

Essential oils used in the poultry industry: Would it be an effective green alternative against *Salmonella* spp. dissemination and antimicrobial resistance?

Heitor Leocádio de Souza Rodrigues, Isis Mari Miyashiro Kolososki, Valdinete Pereira Benevides, Mauro M.S. Saraiva *, Angelo Berchieri Junior *

São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil

ARTICLE INFO

Keywords:
Food safety
Multidrug-resistant
One Health
Phytogenic additives
Salmonellosis

ABSTRACT

Due to the increasing emergence of antimicrobial-resistant strains of *Salmonella* spp. isolated from poultry, and the global trend towards reducing antimicrobial use in food-producing animals, alternatives to these drugs are being sought. Among these alternatives, the antimicrobial action of essential oils (EOs) stands out. These compounds are plant-derived compounds with antimicrobial effectiveness against Gram-positive and Gram-negative bacteria, including strains of *Salmonella*. EOs are employed in the food industry due to their aromatic and antimicrobial properties and their role as natural preservatives as well. However, studies have been approaching their weight gain and performance instead of focusing on the applicability of these compounds in poultry challenged with avian salmonellae or other bacterial diseases. Therefore, the present study aimed to review the pros and cons of using EOs against *Salmonella* spp. in poultry. Although there are reports of the antimicrobial effectiveness of EOs against *Salmonella* spp. both *in vitro* and *in vivo*, their use for this purpose has not been deeply studied. Until then, optimal concentrations for controlling *Salmonella* shedding or toxic concentrations for poultry have not been established. On the other hand, it is known that these products can exhibit synergistic effects with other antimicrobials. Thus, investigations related to the pharmacokinetics, mechanisms of action, and adverse effects of EOs in the poultry's body are important to elucidate the treatment with these alternative antimicrobials, as well as to understand their interactions with both pathogenic bacteria and the bacteria that naturally compose the poultry's microbiota.

1. Introduction

Poultry production is crucial in meeting the nutritional demands for low-cost proteins in various countries (El-Hack et al., 2022). However, the increase in production has led to crowding poultry houses, which facilitates the spread of pathogens, such as those from *Salmonella enterica* (*S. enterica*) specie, which are important from One Health's perspective (Saraiva et al., 2022). Antimicrobials with growth promoters are commonly used in poultry production to control these pathogens and optimize poultry productivity (Zhang et al., 2021a, 2021b). Nonetheless, the emergence of multi-drug-resistant bacteria and the indiscriminate use of these drugs in animal production have prompted the search for new products with similar antimicrobial effects (El-Dayem et al., 2024; Galgano et al., 2023).

Faced with a rising rate of antimicrobial resistance, alternative strategies such as probiotics, prebiotics, symbiotics, organic acids, peptides, and essential oils (EOs) are proposed as alternatives to antimicrobials in broilers (Meenu et al., 2023; Stingelin et al., 2023; Roque-Borda et al., 2022). Among these, EOs are phytobiotics extracted from different plant types and are used as herbal additives in the poultry industry due to their antioxidant, and antimicrobial especially against Gram-negative bacteria, antiviral, and antiparasitic effects (El-Hack et al., 2022; Penteado et al., 2021). These compounds are secondary metabolites extracted from different plant parts, such as flowers, leaves, stems, and roots, composed of various bioactive principles that confer their antimicrobial properties (Meenu et al., 2023).

The mechanism of action of EOs varies according to their active principles (Sousa et al., 2023). Although not fully understood, it is

* Correspondence to: Laboratory of Avian Pathology from the Department of Pathology, Reproduction, and One Health of the Faculty of Agricultural and Veterinary Science at São Paulo State University, Zip Code: 14884-900, Brazil.

E-mail addresses: mauro.saraiva@unesp.br (M.M.S. Saraiva), angelo.berchieri@unesp.br (A. Berchieri Junior).

https://doi.org/10.1016/j.microb.2025.100248
Received 7 October 2024; Received in revised form 10 January 2025; Accepted 22 January 2025
Available online 24 January 2025

known that EOs act on the bacterial cell wall and protein denaturation, in addition to making the membrane permeable to cations such as H^+ and K^+ (Liu et al., 2018; Oliveira et al., 2023). The efficacy of the antimicrobial activity of EOs depends on their composition, and the pathogen in which they act on them as well (Nuñez et al., 2024).

Although there is scientific evidence supporting the antimicrobial activity of these oils (Meenu et al., 2023), research on their use for controlling *Salmonella* spp. infections in poultry is still limited. Most studies using EOs in poultry production focus on promoting zootechnical indices, such as weight gain and maintaining intestinal health in broilers (Huang et al., 2024; Nameghi et al., 2019). Furthermore, there is no standardized dose for the effective or toxic use of EOs for poultry phytotherapy (Hu et al., 2023; Stingelin et al., 2023).

In the poultry's body, EOs are rapidly excreted through the renal pathway after ingestion (Szczepanik et al., 2020). Therefore, their accumulation in the organism is unlikely (Diaz-Sanchez et al., 2015). Although there are few reports of toxicity in poultry, there are reports of embryotoxic effects stimulated by exposure to cinnamaldehyde (Abramovici and Rachmuth-Raizman, 1983).

Based on these observations, we present a deep overview, bringing the pros and cons of the applicability of essential oils as a natural alternative to antimicrobials in poultry production, their potential effects against *Salmonella* genus, and their role in combating antimicrobial resistance.

2. Antimicrobial use in poultry production and antimicrobial resistance

The potential of antimicrobials as growth promoters in poultry farming was first related by Moore et al. in 1946. Since then, in addition to being used to improve zootechnical indicators, these agents have been misused for both prevention of diseases and metaphylaxis purposes (Baptiste and Pokludová, 2020). In 2010, it was estimated that 63,000 tons of antimicrobials were used in animal production. The trend suggests antimicrobial consumption could exceed 100,000 tons by 2030 (Van Boeckel et al., 2015).

The excessive use of antimicrobials in poultry farming has contributed to the selection of resistant strains, a natural evolutionary process accelerated by human intervention (Saraiva et al., 2022). This phenomenon can be attributed to prolonged exposure to these compounds, which may lead to the emergence of resistance genes in both pathogenic microorganisms and commensal microbiota (Xu et al., 2022). One of the factors that correlate poultry farming with the emergence of resistant bacteria is the use of these compounds in sub-therapeutic doses as growth promoters (Saraiva et al., 2018), among the most widely used classes are tetracyclines, penicillins, and aminoglycosides, also used to treat human infections (Rahman et al., 2022). Therefore, these antimicrobials used inappropriately eliminate susceptible microorganisms and select resistant ones, making them predominant in the environment and able to transfer their resistance genes to other bacteria, both within the same species and across distinct species (Saraiva et al., 2022).

The transfer of resistance genes can occur by vertical gene transfer (VGT) or horizontal gene transfer (HGT), through the events of transformation, transduction, and conjugation (Thomas and Nielsen, 2005). Because of these mechanisms, resistant or multi-resistant bacteria are found circulating in domestic and non-domestic species, such as seagulls, turtles, and monkeys (Laborda et al., 2022). Thus, the decline in the effectiveness of antimicrobials has led to the "off-label" use of these drugs in animal production (Caneschi et al., 2023).

Through the consumption of chicken meat and eggs, harboring-resistance genes bacteria can be spread to humans (Saraiva et al., 2022), including pathogens from the *Campylobacter*, *Listeria,* and *Salmonella* genera (Chlebicz and Slizewska, 2018). Especially regarding *Salmonella* spp., reports of resistant strains of poultry origin have been described in the literature (Souza et al., 2020; Moreira et al., 2022; Benevides et al., 2024), including human outbreaks associated with the consumption of poultry meat (CDC, 2018; ECDPC & EFSA, 2022; Gieraltowski et al., 2016).

Faced with the prospect of the emergence of resistance occurring much faster than the development of new antimicrobial molecules (Evangelista et al., 2021) and the difficulty of treating human infections caused by resistant bacteria, the current legislation restricts and rationalizes the use of these drugs in farm animals (Galgano et al., 2023; Nuñez et al., 2024; Oliveira et al., 2023). An example of this practice was the restriction on the use of antimicrobials as growth promoters in the European Union (EU, 2019), a policy that had an economic impact on the EU's main trading partners (Maron et al., 2023). Given the above, there is a clear need to look for alternatives to these drugs (Rahman et al., 2022).

Essential oils, organic acids, competitive exclusion and peptides are proposed alternatives for replacing and reducing the use of conventional antimicrobials in poultry production (Hu et al., 2023; Kolososki et al., 2024; Roque-Borda et al., 2022). As natural alternatives, EOs exhibit antimicrobial effects against significant pathogens (Meenu et al., 2023). Abers et al. (2021) reported that the antibacterial substances derived from lemon, tea tree, and cinnamon EOs demonstrated lower efficacy in eliminating antibiotic-sensitive *S. aureus* in comparison to Methicillin-resistant *S. aureus* (MRSA). This finding suggests that the resistance mechanism of MRSA may confer susceptibility to the antibacterial activity of these EOs. However, the components of frankincense's EO were effective against antimicrobial-sensitive *Pseudomonas aeruginosa* strains and ineffective against resistant strains (Abers et al., 2021).

3. Essential oils

Essential oils are derived from various plants and contain multiple chemical substances, such as terpenes, sesquiterpenes, and aromatic components (Hoffmann, 2020; Meenu et al., 2023). It's estimated that each oil contains between 20 and 60 chemical components, but some EOs may contain more than 300 (Sousa et al., 2023). However, typically two or three chemical substances are more frequently concentrated than the others in EOs (Aziz et al., 2018), for instance: basil EO has p-allylanisole as a major component, concentrated at approximately 70 %, and linalool at 18 % (Nuñez et al., 2024); thyme EO, contains thymol concentrated at approximately 47 %, p-cymene at 20 %, and ϒ-terpinene at 9 % (Galgano et al., 2023). In general, the evaluation of the constituents of different EOs is performed using Gas Chromatography-Mass Spectrometry (GCMS) (Aziz et al., 2018).

In different countries and cultures, EOs have been employed due to their therapeutic and aromatic effects, particularly within traditional medicine practices (Hoffmann, 2020). In plants' physiology, EOs play a role in antimicrobial protection and attracting or repelling insects due to their aromatic properties (Bakkali et al., 2008). Due to this antimicrobial capability, these compounds are considered potentially effective against pathogens (Sousa et al., 2023) and have passed to be tested against bacteria from food-production animals.

Historically, the extraction of these products was first performed by Arabs during the Middle Ages using the hydrodistillation method (Bakkali et al., 2008). The extraction process determines the quality of the product and composition of EOs; improper extraction can interfere with the chemical and pharmacological activity of the respective components (Aziz et al., 2018). Currently, hydrodistillation remains the most widely used method for industrial EO extraction (Ferrentino et al., 2020), though alternative methods such as steam distillation, hydrodiffusion, solvent extraction, and microwave-assisted extraction are also employed (Aziz et al., 2018).

To optimize the efficacy of EOs and preserve their bioactive components, the pharmaceutical and food industries have adopted microencapsulation or nanoencapsulation technologies (Movahedi et al., 2024; Linh et al., 2022). The primary goal of this technique is to protect these bioactives from oxidative reactions, degradation, and volatilization (Jia et al., 2016). Encapsulated products allow for broader release in the gastrointestinal tract due to the matrices used in encapsulation (Mo et al., 2022; Movahedi et al., 2024). In previous tests involving microencapsulated EOs for poultry, commonly used matrices included maltodextrin, lipid matrices of palm oil, hydrogenated vegetable fat, and liposomes (Moharreri et al., 2022; Meligy et al., 2023; Stingelin et al., 2023; Wang et al., 2019; Zhang et al., 2019), as well as encapsulation via the spray-drying method (Mo et al., 2022; Moharreri et al., 2022).

There are various methods for microencapsulating EOs, each with their own advantages and disadvantages depending on the desired objectives. Coacervation, spray drying, and interfacial polymerization are some of the techniques employed (Sundar & Parikh, 2023). However, methods involving thermal processes present contribute to the volatilization of the volatile constituents present in EOs (Cimino et al., 2021).

4. Antimicrobial and synergic effects of essential oils

The antibacterial effect varies with each EO and each bactericidal substance they contain. These substances are volatile constituents naturally responsible for the antimicrobial defense of plants (Abers et al., 2021), as discussed above. The use of certain EOs and their components is classified as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration (FDA) (Oliveira Filho et al., 2023; Silva et al., 2012).

The visualization of bacterial morphology allows for understanding the probable mechanisms of action of EOs (Sousa et al., 2023). For instance, after exposure to *Citrus medica* L. EO, *Escherichia coli* and *Staphylococcus aureus* showed holes on the cell surface and a shriveled appearance (Li et al., 2019). In contrast, exposure to mustard essential oil resulted in holes in the cell wall and deformity of the structure in *E. coli* (Turgis et al., 2009). In addition to alterations in the plasma membrane and cell wall (Li et al., 2019), other observed effects include a decrease in bacterial ATP production (Turgis et al., 2009), changes in bacterial protein synthesis (Burt et al., 2007) and gene modulation (Frazzon et al., 2018), as illustrated in Fig. 1. Even though the bactericidal effect of EOs is damage to the bacterial cell wall, the type of damage to the cell wall can vary in each bacterium species (Turgis et al., 2009). Despite these findings, the exact mechanisms further research is needed to fully elucidate the specific effects, and their specific mechanisms, of EOs on bacterial cell structures.

One of these substances of EOs with bactericidal properties is carvacrol, a compound found in EOs obtained from various aromatic plants such as oregano (*Origanum vulgare*) and marjoram (*Origanum majorana*) (Bona et al., 2012; Silva et al., 2012). During *in vitro* experiments, carvacrol has been shown to be an effective antibacterial agent against *E. coli* and *S. enterica* serovar Typhimurium (*S.* Typhimurium) (Severino et al., 2015). Still regarding *E. coli*, it was observed that carvacrol interfered with flagellin synthesis, rendering the bacteria aflagellate, which compromises bacterial motility and diminishes its pathogenicity (Burt et al., 2007).

Thymol is a substance present in the essential oil of some plants such as thyme (Meenu et al., 2023). A phenolic compound within the monoterpene group, it is extensively utilized in the food industry for its efficacy as a natural preservative (Barbosa et al., 2020). Its antibacterial effect on *Listeria monocytogenes* and some serovars of *S. enterica* (Enteritidis, Typhimurium, Derby, Heidelberg, Livingstone, Paratyphi, and others), have been observed both *in vitro* and *in vivo* (Bona et al., 2012; Meenu et al., 2023). Besides its antibacterial effect, thymol is also used for its antioxidant properties (Barbosa et al., 2020).

Another EO compound, eugenol is included in the group of phenylpropenes, along with cinnamaldehyde, and is extracted respectively from cloves (Oliveira et al., 2020) and cinnamon (Zhang et al., 2022). Both exhibit broad antibacterial activity, being more effective against

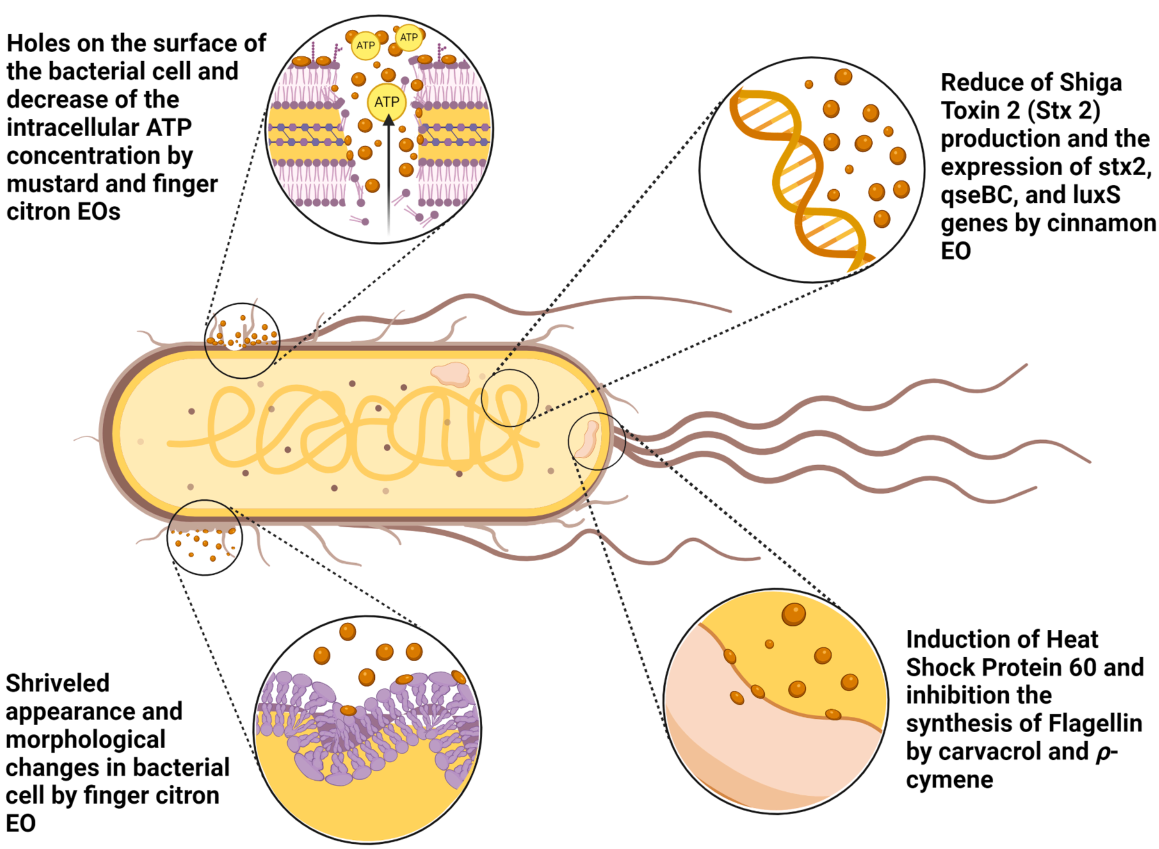

Fig. 1. Main alterations reported in bacterial cells due to contact with essential oils and their chemical components, according to Burt et al. (2007); Frazzon et al. (2018); Li et al. (2019); Turgis et al. (2009). This figure was created using BioRender.com.

Gram-negative bacteria (Meenu et al., 2023). The bactericidal mechanism of cinnamaldehyde against *E. coli* involves disruption of the cell wall and induction of oxidative stress, leading to bacterial cell death (Visvalingam et al., 2017). In addition, eugenol and cinnamaldehyde also possess antifungal activity and are commercially used as natural antimicrobial agents (Oliveira Filho et al., 2023).

EOs have the potential to exhibit synergistic effects with different products, such as antimicrobials, organic acids, or other EOs (Hu et al., 2023; Rapper et al., 2023). Combined with another antimicrobial agent, they can synergize against multidrug-resistant bacteria and significantly reduce their Minimum Inhibitory Concentration (MIC) (Meenu et al., 2023).

The synergism of some EOs has been highlighted in the effect on bacteria. Cinnamaldehyde, when combined with antimicrobials, increased the susceptibility of erythromycin-resistant *E. coli* and maximized the effect of tetracycline and novobiocin (Visvalingam et al., 2017). Organic acids combined with EOs acted synergistically against *Salmonella* Enteritidis in the study by Hu et al. (2023). The combination of tea tree with oregano EOs enhanced the antimicrobial effect of both oils against respiratory pathogens (Rapper et al., 2023). Thus, combining different EOs or other products can further enhance the antimicrobial effect of EOs (Meenu et al., 2023). Alternatively, EOs can be synergistically combined with probiotic bacteria to enhance antimicrobial activity against *Salmonella enterica* strains (Bartkiene et al., 2020).

5. Efficacy of essential oils against *Salmonella* spp. and other pathogens in poultry

Due to their safety for consumption on feed and antimicrobial activity against important poultry pathogens (El-Shall et al., 2020; Mariotti et al., 2022; Puvaca et al., 2020; Shayeganmehr et al., 2018; Zhang et al., 2022), EOs are likely candidates to replace antibiotic therapies in poultry (Puvaca et al., 2020) and preserve the antimicrobials efficiency. However, the main challenge for using EOs to replace antimicrobials in poultry is that the concentration of active principles, when reaching the intestine, is lower than the MIC set for them (Horky et al., 2019). Although their antimicrobial action is proven, little is known about the non-toxic recommended concentrations for different oils and their mechanisms of action.

Although there are several studies on the *in vitro* antimicrobial efficacy of different essential oils against different serovars of *Salmonella* spp. (Barbosa et al., 2020; Boy et al., 2023; Porter et al., 2020; Robinson et al., 2022; Somrani et al., 2022), few have investigated the potential application of EOs in the treatment of avian salmonellosis, as we disposable de major in Table 1. Overall, cinnamon, clove, and oregano EOs are the most effective antimicrobial agents both *in vitro* and *in vivo* against salmonellosis and other avian pathogens (Barbosa et al., 2020; Ebani et al., 2018; Ebani et al., 2019; Meligy et al., 2023; Stingelin et al.; 2023).

In a recent study, essential oils were combined with organic acids in the diet of broiler chickens experimentally infected with *S.* Heidelberg, *S.* Minnesota, and *S.* Typhimurium (Stingelin et al., 2023). A reduction in bacterial load was observed on the 7th and 14th days post-infection in the treated groups (Stingelin et al., 2023). A similar experiment has been carried out using a combination of organic acids with EOs, which not only reduced *S.* Enteritidis, but also facilitated enhanced weight gain among the treated broilers (Hu et al., 2023). However, not all studies were able to achieve similar results, highlighting the divergence between research. For instance, the same antimicrobial effect has not been observed against infections by *S.* Enteritidis and *C. jejuni* in experimentally infected chickens (Girard et al., 2024).

The cecum is recognized as the primary locus of bacterial proliferation during paratyphoid infections in poultry (Barrow et al., 2015). Therefore, in studies aiming to control *Salmonella* paratyphoid infections with EOs, it is crucial to ensure that the active principles of the oils reach the desired concentrations at this location. In a recent study, Stingelin et al. (2023) indicated that microencapsulation of the oils allowed for a reduction of *S.* Minnesota, *S.* Heidelberg, and *S.* Typhimurium in the cecum of broilers. Encapsulation of the oils facilitates better release of

Table 1
Antimicrobial effects of essential oils against *Salmonella* spp. *in vitro* and *in vivo*.

Essential oil	Actives substances identified	Serovar	Concentration	Used methods	Authors
Cinnamon and clove	Cinnamaldehyde, eugenol and others	*S.* Enteritidis and *S.* Typhimurium	1.26–0.63 and 2.63–0.16 mg/mL	MIC and DDST	Ebani et al. (2019)
Savory	Thymol, terpinene, p-cymene, and others	*S.* Enteritidis	0.31–0.62 μL/mL	MIC	Seyedtaghiya et al. (2021)
EOs components and organic acids	Carvacrol, thymol, cinnamaldehyde, and others	*S.* Enteritidis	800 mg/kg	Experimental infection in broilers chicks	Zhang et al. (2019)
Laurel and zahter	Carvacrol, thymol, terpinene, 1,8-cineole, terpinyl, and others	*S.* Typhimurium	2 mg/mL and 0.2 μL/mL	MIC	Yilmaz et al. (2024)
Oregano, rosemary, and EOs components	Carvacrol, 1,8-cineole	*S.* Enteritidis	1,25 μL/mL and 20 μL/mL	MIC	Cariri et al. (2019)
Thyme, savory, peppermint, black pepper	Thymol, carvacrol, p-cymene, terpinene, and others	*S.* Enteritidis	1.87 mg/mL	MIC	Moharreri et al. (2022)
Clove	Eugenol and others	*S.* Enteritidis	0.32–0.64 mg/mL	MIC and DDST	Somrani et al. (2022)
Oil mix	Carvacrol, limonene, menthol, cineol, eugenol, and others	*S.* Typhimurium	0.10–1 % v/v	BMD and MIC	Mariotti et al. (2022)
Oil mix	Eucalyptol, pinene, limonene, thymol, and others	*S.* Infantis and *S.* Typhimurium	40 μL/mL	BMD and MIC	Di Vito et al. (2020)
Flowers of Dehesa of Extremadura	Phenolic compounds	*S.* Choleraesuis	10–100 μL/mL	DDST	Boy et al. (2023)
Oregano	Thymol, carvacrol, and others	*S.* Enteritidis	130 μg/mL	MIC	Barbosa et al. (2020)
Oil mix and organic acids	Thymol, carvacrol, cinnamaldehyde, butyric acid, and others	*S.* Enteritidis	500–800 mg/kg	Experimental infection in broilers chicks	Hu et al. (2023)
Oil mix and organic acids	Thymol, carvacrol, and sorbic acid	*S.* Heidelberg, *S.* Minnesota and *S.* Typhimurium	1–2 g/kg	Experimental infection in broilers chicks	Stingelin et al. (2023)
Lippia origanoides	Carvacrol, thymol, diethylphenol, and others	*Eimeria* and *S.* Typhimurium	37 ppm	Experimental infection in broilers chicks	Coles et al. (2021)

Legend: Minimum Inhibitory Concentration (MIC); Minimum Bactericidal Concentration (MBC); Disk Diffusion Susceptibility Test (DDST); Broth Microdiluition Susceptibility Testing (BMD); Parts Per Million (PPM).

these compounds throughout the gastrointestinal tract and increases their bioavailability, ensuring desired concentrations in the organism (Mo et al., 2022). However, the antimicrobial efficacy of the oils depends not only on the encapsulation of the product but also on the concentration of the active principle and the microorganism (Hu et al., 2023).

In a manner analogous to microencapsulation, nanoencapsulation enhances the bioavailability of phytobiotics through small capsules (Movahedi et al., 2024). Generally, capsules are made from biological materials that will be degraded at specific locations within the organism, such as the duodenum (Linh et al., 2022). Encapsulation techniques can also amplify the antimicrobial effect of EOs. In another study, lavender EO demonstrated three times its antimicrobial potency against *S. aureus* (Yuan et al., 2019).

Recommended dosages of EOs to control *Salmonella* spp. or other avian bacterial pathogens have not been established, and there is variation in suggested dosages across different studies (Bona et al., 2012; Hu et al., 2023; Stingelin et al., 2023). Products based on a mix of EOs and organic acids shown efficacy in reducing *S.* Enteritidis in the cecum using concentrations of 500 mg/kg of feed and 800 mg/kg (Hu et al., 2023), while the concentrations for reducing the excretion of *Salmonella* serovars were found to be 1 g/kg and 2 g/kg of feed (Stingelin et al., 2023). On the other hand, Bona et al. (2012), reported a compound with a minimum guarantee of 4.5 mg/kg of carvacrol, 1 g/kg of cinnamaldehyde, 2 g/kg of cineol, and 20 g of capsaicin per kg of feed was used against *S.* Enteritidis and other bacteria.

Despite the majority of EOs having no established reliable concentrations, the European Food Safety Authority (EFSA) published, in 2019, a report containing the maximum safe concentrations of oregano essential oil for production animals: 22 mg/kg of feed for broilers, 33 mg/kg for laying hens, and 30 mg/kg for turkeys (EFSA et al., 2019). However, considering the variety of EOs and their different active principles, further research is needed to investigate the effective and tolerable doses of these substances in the poultry industry. Although some oils demonstrate *in vitro* antimicrobial activity, not all are recommended for *in vivo* tests due to their minimum inhibitory concentration being higher than acceptable levels in animal production, cost-effectiveness, and palatability (Di Vito et al., 2020).

Even in the absence of a report establishing adequate concentrations of other EOs, some studies documented antimicrobial effectiveness against avian pathogens at non-toxic doses for poultry. For instance, the administration of 100 mg of tea tree EO per kg of feed resulted in a significant reduction in *Mycoplasma synoviae* infection in laying hens (Puvaca et al., 2020). Additionally, a concentration of 0.5 mL of carvacrol and thymol per liter of water reduced *C. jejuni* excretion and increased weight gain in experimentally infected broilers (El-Dayem et al., 2024). The inclusion of 37 ppm of *Lippia origanoides* EO in the diet of experimentally infected broilers resulted in reduced excretion of *S.* Typhimurium, *Eimeria maxima*, and a decrease in the occurrence of necrotic enteritis by *C. perfringens* (Coles et al., 2021).

In addition to their bactericidal effect, EOs possess virucidal properties (El-Shall et al., 2020). Against Avian Infectious Bronchitis Virus (IBV), using 1 and 2 mL of a mixture of EOs and cinnamaldehyde per liter of water resulted in a significant reduction in viral load and increased cure rate in broilers (Zhang et al., 2022). The concentration of 0.5 mL of the mixture of oils (50 g of oregano EO, 10 g of carvacrol, 33 g of thyme, 50 g of eucalyptus EO, 5 g of thymol, and 10 g of eucalyptol) added to 1 L of water reduced Newcastle Disease Virus excretion and decreased poultry mortality (El-Shall et al., 2020).

Another use of EOs in the poultry chain is in the meat industry, as antimicrobials and natural preservatives, aiming to extend the shelf life of products (Andrade et al., 2023). Alternatively, some combinations of EOs have been used to reduce *Salmonella* spp. in poultry meat products. Studies by Porter et al. (2020) and Robinson et al. (2022) evaluated the addition of different EOs to chicken meat to reduce contamination by *Salmonella* spp. Using a formulation made from white mustard essential oil and carvacrol, a reduction of 0.7 log CFU/g of *S.* Enteritidis, *S.* Typhimurium, *S.* Heidelberg, *S.* Kentucky, and *S.* Montevideo was observed in chicken meat (Porter et al., 2020). In the combination of garlic and ginger oils, a reduction of 2 log CFU/g of *S.* Infantis, *S.* Enteritidis, and *S.* Typhimurium was also observed in chicken meat (Robinson et al., 2022).

A limitation observed in experimental studies involving essential oil treatments is the lack of description regarding the sanitary condition of the environment where the broilers were housed. After all, sanitary conditions are closely linked to the success of antimicrobial treatment (Zhai et al., 2018).

The antioxidant properties of EOs provide benefits when incorporated into meat products, particularly poultry, which is prone to oxidative deterioration due to high concentrations of polyunsaturated fatty acids (Zhai et al., 2018). It was observed that the inclusion of lemongrass and pedestrian EOs in the diet of broilers improved the oxidative stability of the thigh-drumstick meat during storage (Azevedo et al., 2021).

Although these products can be added to foods, the use of EOs in foods can impart desirable or undesirable odors and flavors depending on the type and concentration of oil used (Zhai et al., 2018). As a disadvantage, the alteration in flavor and odor can affect the product's acceptability by the consumer (Ferrentino et al., 2020), even though some reports are conflicting. For instance, although, the concentrations capable of eliminating or reducing foodborne bacteria can directly affect sensory acceptability by humans (Meira et al., 2017), the use of essential oils associated with the diet of laying hens did not interfere with the nutritional and sensory quality of the eggs (Puvaca et al., 2020).

Even though there are reports of the therapeutic efficacy of EOs against Gram-negative and Gram-positive bacteria (Fig. 2), both the dysbiotic and toxic effects of these treatments in poultry are not frequently reported in some studies. Recent report showed a reduction in the population of *Bifidobacterium* spp. and *Lactobacillus* spp., alongside an increase in *Campylobacter* spp., was observed in broilers experimentally infected with *S.* Enteritidis and treated with a mixture of garlic, lemon, thyme, and eucalyptus EOs (Laptev et al., 2021). Conversely, in the study by Girard et al. (2024), the authors demonstrated that treatment with EOs in broilers experimentally infected simultaneously with *S.* Enteritidis and *C. jejuni* improved the cecal and ileal microbiota composition of the broilers. It is believed that the beneficial effect of EOs on the cecal and ileal microbiota can be attributed to the promotion of competitive exclusion (Girard et al., 2024).

6. Essential oils interactions on poultry's organism

Although the chemical composition of some oils is well known, the pharmacokinetics of these components and their action remain unclear, and when there is information, these data are based on studies conducted in mammals (Čabarkapa et al., 2020). The literature states that pharmacokinetics is related to absorption in the animal's tissues and is an important topic regarding the possibility of intoxicating the poultry and altering the sensory aspects of the meat intended for human consumption (Ocel'ová et al., 2016). Sensory changes are directly related to the potential to prevent lipid oxidation that some compounds in EOs exhibit, resulting in alterations to the food's chemical properties (Kumar et al., 2015; Ruiz-Hernández et al., 2023).

In poultry, EOs are characterized by rapid metabolism and elimination (Zhai et al., 2018). After absorption, the terpenoids that makeup EOs are eliminated by the kidneys in the form of glucuronides or exhaled as CO_2 (Szczepanik et al., 2020). The predominant method of administration is oral, typically delivered through drinking water or feed (Puvaca et al., 2020; Zhang et al., 2022). Another alternative route for the administration of EOs is through the inhalation of chemical components by adding the oil to poultry litter (Sniegocki et al., 2022), however less common. In the gastrointestinal tract, these components interact with food and are quickly digested in the small intestine along

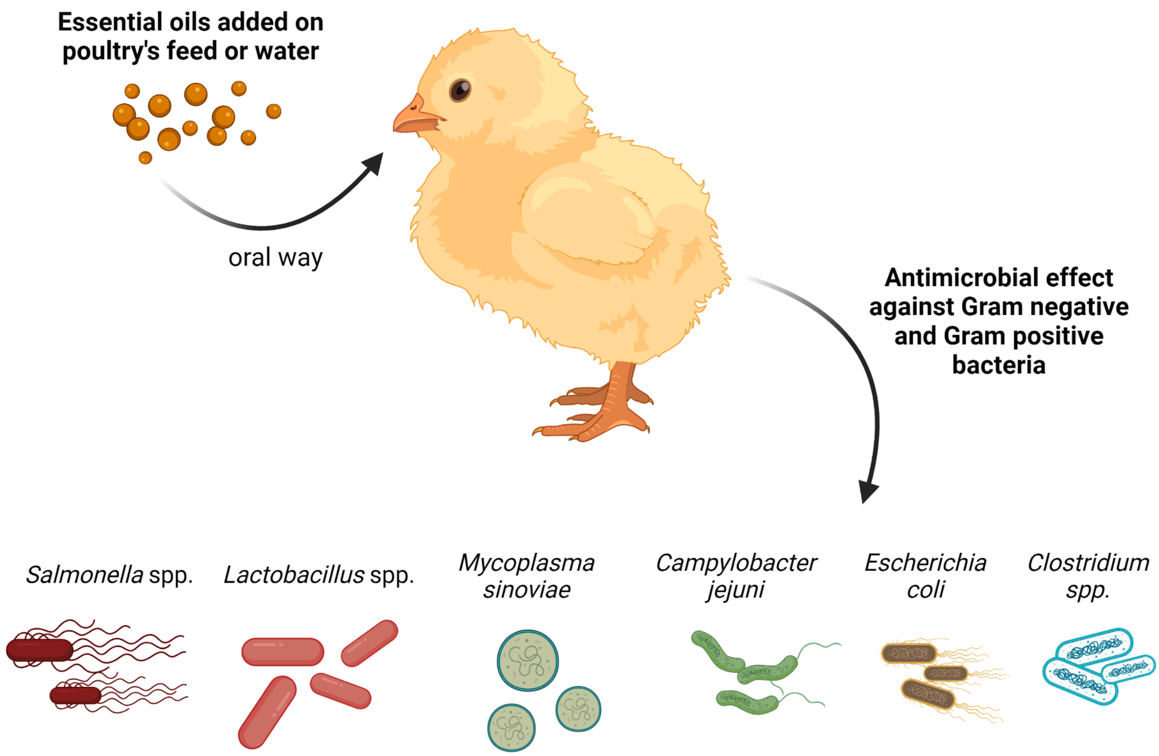

Fig. 2. Antimicrobial effect of EOs added on poultry's feed or water against Gram-positive and Gram-negative bacteria. This figure was created using BioRender.com.

with other dietary lipids and are metabolized in the liver after a first-pass effect (Horky et al., 2019), as illustrated in Fig. 3. During absorption, EOs can increase the production of digestive secretions and alter the microbial composition of the intestine, favoring symbiotic species and controlling pathogens (Attia et al., 2019; Gadde et al., 2017; Puvaca et al., 2020). The quantification of the chemical components of EOs absorbed by the poultry can be performed using liquid chromatography-mass spectrometry from samples of liver, muscle, plasma, and lung tissue (Sniegocki et al., 2022).

Moreover, studies indicate that the use of EOs can improve both the

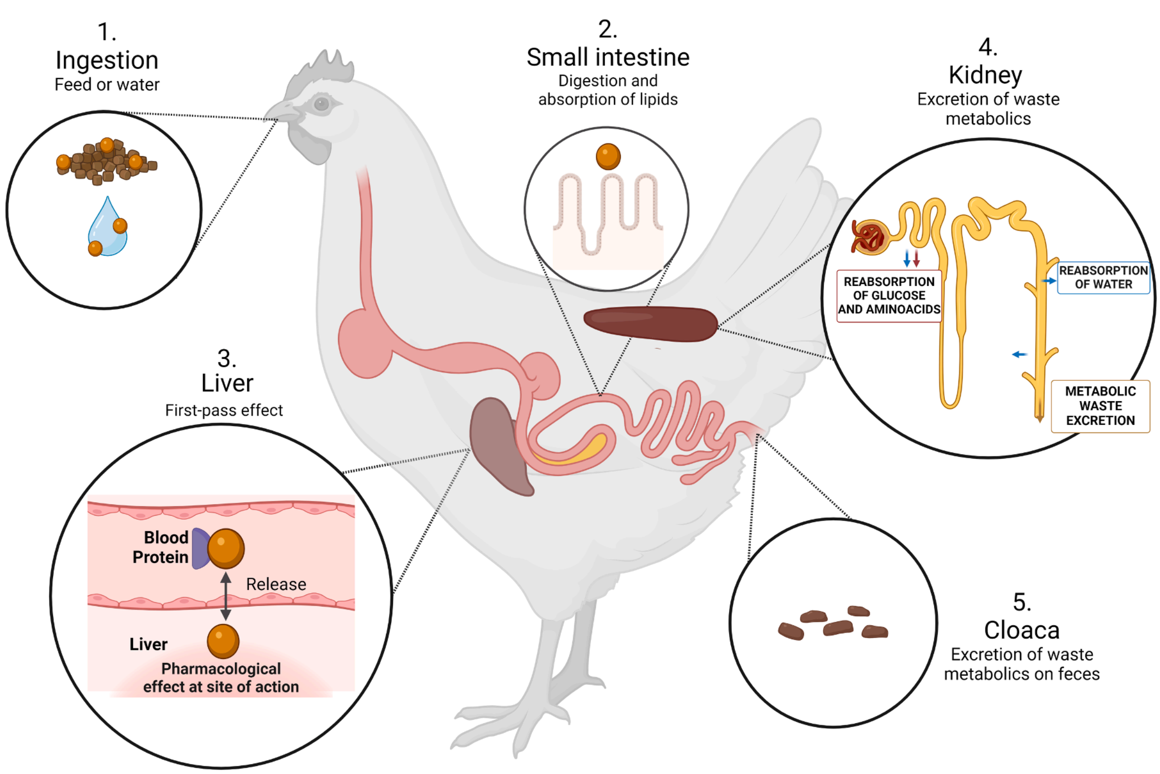

Fig. 3. Probable pharmacokinetic pathways of essential oils in the chicken organism, according to Attia et al. (2019); Gadde et al. (2017); Horky et al. (2019); Puvaca et al. (2020). This figure was created using BioRender.com.

cellular and humoral immune response (Nameghi et al., 2019). For example, an increase in serum titers of anti-Avian Infectious Bronchitis (IB) antibodies was observed in broilers treated with EOs. Similarly, the production of antibodies in vaccination programs can be enhanced in broilers treated with EO. However, in other studies, such as that by El-Shall et al. (2020), treatment with EOs during immunization against Avian Influenza (AI) and IB did not affect the poultry's immune response.

Although most research is focused on investigating the antimicrobial properties of EOs against different pathogens, issues such as toxicity and pharmacological interactions warrant further investigation (Sousa et al., 2023). Despite their rapid elimination from the body, reports have documented toxicity associated with the consumption of high concentrations of EOs, including hepatotoxicity, nephrotoxicity, and vascular changes (Oliveira et al., 2023). Additionally, the literature infrequently reports other adverse effects, such as respiratory disorders, dermal irritation, potential carcinogenicity, and reproductive toxicity (Horky et al., 2019). For instance, Beier et al. (2014) identified hepatomegaly and elevated serum aspartate aminotransferase in poultry treated with 5 % linalool, a compound found in basil oil. Adverse effects in humans, such as oral irritation and allergy, have also been linked to the phenols in clove cinnamon EOs (Triviño et al., 2019). In mice showed no mortality at high doses (200 mg/kg body weight) of oregano essential oil, with the primary effect being feed rejection due to the high concentration of the oil (EFSA et al., 2019).

However, when compared to antimicrobials, high doses of phytobiotics present a lower risk of poisoning at high doses (Horky et al., 2019). In addition, according to Linh et al. (2022), microencapsulation may increase the bioavailability and improve the physicochemical stability of active ingredients. In this way, the microencapsulation technique can be a solution to overcome some of the limitations of EO, such as the risk of intoxication, low water solubility, high volatility, and degradation by light (Mo et al., 2022).

7. Other uses of essential oils in the poultry industry

In addition to their use in pathogens control in poultry, EOs may perform other benefits in poultry management due to their antimicrobial properties (Galgano et al., 2023). EOs possess the capability to reduce bacterial contamination at critical points within the poultry environment, such as air, equipment, bedding, facilities, poultry, and eggs (Xu et al., 2022). These products can be used as sanitizers in poultry houses and equipment, ensuring they do not pose health risks to poultry (Oliveira et al., 2024). In the study by Ponomarenko et al. (2021), a 99 % reduction in bacterial load in the air of a poultry house was observed due to the daily use of thyme, fir, and eucalyptus EOs aerosol, without causing toxicity to the animals.

One of the uses of EOs in the poultry industry is to reduce bacterial load on the chicken warehouses. Therefore, studies investigated methods to control the bacteria both in the poultry and the poultry litter to interrupt the epidemiological cycle of paratyphoid salmonellosis (Galgano et al., 2023; Hu et al., 2023; Penha Filho et al., 2010). Experimental data showed that the application of thyme essential oil (EO) was effective in reducing bacterial contaminants within poultry litter, such as *E. coli*, *S.* Derby, and *Mammaliicoccus lentus* (Galgano et al., 2023). In addition to its antibacterial effect, the addition of oregano EO and carvacrol to poultry litter can also eliminate *Alphitobius diaperinus* larvae within 24 h (Szczepanik et al., 2020).

A strategic point for optimizing production and hatchability is the sanitization of fertile eggshells (Tebrün et al., 2020). Recently, some studies have proposed EOs as a natural alternative to the sanitizers already used for this purpose (Mustafa et al., 2023; Oliveira et al., 2023). However, some components of EOs, such as thymol, can cause embryonic lesions depending on their application (Oliveira et al., 2024). In the study by Mustafa et al. (2023), lavender EO was used to sanitize fertile eggs, reducing aerobic bacteria and *Salmonella* spp. compared to other sanitizers.

In another study, the physical quality of chicks from eggs sanitized with clove EO was compared to those sanitized with paraformaldehyde, showing that phytogenic sanitization was superior in quality and did not cause toxicity in poultry (Oliveira et al., 2020). However, some embryonic alterations, such as yolk sac retention, reduced hatchability, and embryonic death, were observed in embryos from fertile eggs sanitized with EOs (Tebrün et al. 2020). Other studies reported that carvacrol and citral, when injected into eggs, caused embryonic malformations and delayed embryonic development (Ali et al., 2019; Zhang et al., 2021a, 2021b). Therefore, the administration method and chemical composition can interfere with embryonic quality (Oliveira et al., 2023).

Phytogenic additives are also proposed as alternatives to conventional growth promoters for poultry. These are divided into four groups: herbs, botanicals, EOs, and oleoresins (El-Hack et al., 2022). The effectiveness of EO-based growth promoters is like that of antimicrobials (Islam et al., 2024). Alternative products to antibiotics are a global trend to reduce antimicrobial resistance while maintaining poultry productivity (Huang et al., 2024). Moreover, some phytogenic additives are still economically unviable for large-scale use.

Intestinal health is a critical point in broiler chicken nutrition (Huang et al., 2024). Various studies have investigated the benefits of thymol, carvacrol, and EOs on intestinal health and their impact on the avian microbiota (Islam et al., 2024; Huang et al., 2024; Nameghi et al., 2019). It is believed that phytogenic additives can enhance digestibility, nutrient absorption, and the reduction of pathogenic microorganisms (Ocel'ová et al., 2016). Liu et al. (2018) reported the effect of carvacrol dosages on the nutritional performance and intestinal health of poultry was analyzed. Groups treated with 300–400 μL of carvacrol showed increased sucrase, lactase, and other enzyme activity, as well as an increase in goblet cell content and a reduction in *Salmonella* spp. and *E. coli* (Liu et al., 2018).

In addition to improving performance, thymol and carvacrol reduced the intestinal lesion score in broiler chickens (Islam et al., 2024). The reduction of intestinal lesions by EO treatment can be attributed to their effectiveness in controlling *C. perfringens* and *Eimeria maxima* (Coles et al., 2021; Gharaibeh et al., 2021; Pham et al., 2022). The control of *C. perfringens*, *E. maxima,* and other enteric pathogens and the selection of beneficial bacteria contribute to maintaining the intestinal health of poultry and, consequently, to the reduction of enteric lesions (Nameghi et al., 2019). In addition to preventing intestinal colonization by pathogenic bacteria, EOs can be combined with certain probiotics, such as *Saccharomyces cerevisiae* (Ebani et al., 2019). However, some oils, such as thyme oil, may inhibit probiotic bacterial strains, including those from the *Lactobacillus* group. Despite this inhibition of beneficial bacteria, their metabolites can exhibit antibacterial activity against strains of *Salmonella enterica* (Bartkiene et al., 2020).

8. Final considerations and future perspectives

As natural substances with antimicrobial efficacy against various serovars of *Salmonella enterica*, EOs are proposed as alternatives to conventional antimicrobials. However, to ensure the safe use of these products *in vivo*, more research is needed to investigate effective and toxic concentration thresholds in broilers and laying hens. Some observed limitations include the scarcity of literature on the *in vivo* control of *Salmonella* spp. using EOs and the cost-benefit relationship of these products for this purpose. Therefore, future research should focus on investigating the interactions between phytobiotics, poultry, and the target bacteria to enhance antimicrobial efficacy. Zootechnical information, such as daily intake and its impact on poultry development, are critical considerations for future research involving *in vivo* experiments with phytobiotics. In the long term, regulation by official agencies regarding the use of these products as antimicrobials in poultry farms will be essential tools for rationalizing and properly managing their application.

CRediT authorship contribution statement

Kolososki Isis Mari Miyashiro: Writing – original draft, Methodology, Investigation, Conceptualization. **Benevides Valdinete P.:** Writing – review & editing, Writing – original draft, Validation, Investigation. **Rodrigues Heitor Leocádio de Souza:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Saraiva Mauro M. S.:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Data curation. **Berchieri Junior Angelo:** Writing – review & editing, Writing – original draft, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by São Paulo Research Foundation (FAPESP - grant number 2024/03279-0). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Finance Code 001.

Data availability

Data will be made available on request.

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