UNIVERSIDADE ESTADUAL PAULISTA – UNESP
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS – CÂMPUS
DE JABOTICABAL

AVALIAÇÃO DA ADIÇÃO DE ÓLEOS ESSENCIAIS NA DIETA DE FRANGOS DE CORTE VISANDO O CONTROLE DA COLONIZAÇÃO INTESTINAL POR *Salmonella* Enteritidis

Heitor Leocádio de Souza Rodrigues
Médico Veterinário

2026

UNIVERSIDADE ESTADUAL PAULISTA – UNESP
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS – CÂMPUS DE JABOTICABAL

AVALIAÇÃO DA ADIÇÃO DE ÓLEOS ESSENCIAIS NA DIETA DE FRANGOS DE CORTE VISANDO O CONTROLE DA COLONIZAÇÃO INTESTINAL POR *Salmonella* Enteritidis

Discente: Heitor Leocádio de Souza Rodrigues
Orientador: Prof. Dr. Angelo Berchieri Junior
Coorientadora: Drª. Valdinete Pereira Benevides

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Ciências Veterinárias.

2026

R696a

Rodrigues, Heitor Leocádio de Souza
Avaliação da adição de óleos essenciais na dieta de frangos de corte visando o controle da colonização intestinal por Salmonella Enteritidis / Heitor Leocádio de Souza Rodrigues. -- Jaboticabal, 2026
93 p. : il., tabs.

Dissertação (mestrado) - Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal
Orientador: Angelo Berchieri Junior
Coorientadora: Valdinete Pereira Benevides

1. Salmonella Enteritidis. 2. Óleos essenciais. 3. Indústria avícola. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Dados fornecidos pelo autor(a).

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: AVALIAÇÃO DA ADIÇÃO DE ÓLEOS ESSENCIAIS NA DIETA DE FRANGOS DE CORTE VISANDO O CONTROLE DA COLONIZAÇÃO INTESTINAL POR *Salmonella* Enteritidis

AUTOR: HEITOR LEOCADIO DE SOUZA RODRIGUES

ORIENTADOR: ANGELO BERCHIERI JUNIORCOORIENTADORA: VALDINETE PEREIRA BENEVIDES

Aprovado como parte das exigências para obtenção do Título de Mestre em Ciências Veterinárias, área: Saúde Única pela Comissão Examinadora:

Documento assinado digitalmente
gov.br VALDINETE PEREIRA BENEVIDES
Data: 27/01/2026 10:28:18-0300
Verifique em https://validar.iti.gov.br

Pós-doutoranda VALDINETE PEREIRA BENEVIDES (Participaçao Virtual)
Departamento de Patologia, Reprodução e Saúde Única / FCAV UNESP Jaboticabal

Documento assinado digitalmente
gov.br MARCIANE MAGNANI
Data: 27/01/2026 10:54:49-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. MARCIANE MAGNANI (Participaçao Virtual)
Departamento de Engenharia de Alimentos / Universidade Federal da Paraíba (UFPB) - João Pessoa/PB

Documento assinado digitalmente
gov.br OLIVEIRO CAETANO DE FREITAS NETO
Data: 27/01/2026 11:52:40-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. OLIVEIRO CAETANO DE FREITAS NETO (Participaçao Virtual)
Departamento de Medicina Veterinária Preventiva / Universidade Federal de Minas Gerais (UFMG) - Belo Horizonte/MG

Jaboticabal, 27 de janeiro de 2026.

DADOS CURRICULARES DO AUTOR

Heitor Leocádio de Souza Rodrigues – nascido no município de Patrocínio, Minas Gerais, em 16 de maio de 2001, filho de Rosana Cristina Pereira de Souza Rodrigues e Paulo César Rodrigues. Bacharel em Medicina Veterinária pela Universidade Federal de Uberlândia (UFU) no ano de 2024. Durante a graduação, foi pesquisador bolsista de Iniciação Científica fomentado pela Fundação de Amparo à Pesquisa do Estado de Minas Gerais (2022-2023) e monitor nas disciplinas de Bioquímica (2019), Doenças Bacterianas dos Animais Domésticos (2023) e Ornitopatologia (2023). Em 2024, ingressou no mestrado pelo programa de “Ciências Veterinárias” da FCAV/Unesp como pesquisador bolsista fomentado pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), sob orientação do Prof. Dr. Angelo Berchieri Junior e coorientação da Drª. Valdinete Pereira Benevides. Já em novembro de 2025, foi contratado pela Seara/JBS para atuar como “Médico Veterinário Sanitarista I – Responsável Técnico” no Laboratório de Saúde Animal (LSA) na unidade de Seara/SC.

EPÍGRAFE

"Somos feitos da mesma matéria da qual são feitos os sonhos"

— William Shakespeare

DEDICATÓRIA

Dedico este trabalho a todos que já se foram, aos que permanecem e aos que ainda virão — especialmente àqueles que contribuíram para a construção do meu ser.

AGRADECIMENTOS

Aos meus pais — Paulo César Rodrigues e Rosana Cristina Pereira de Souza Rodrigues — pela criação, pelo amor, pela parceria, pelo incentivo e, especialmente, pela dádiva da vida.

À minha família, aos amigos, ao Laboratório de Ornitopatologia — a todos integrantes — e à Universidade Estadual Paulista “Júlio de Mesquita Filho” pelo apoio recebido durante minha passagem por aqui. Vocês foram e são essenciais para mim.

Ao professor Dr. Angelo Berchieri Junior e a Drª. Valdinete Pereira Benevides, agradeço pelas orientações prestadas, pela paciência e pelas oportunidades que me foram concedidas. Sem os senhores, eu não chegaria até aqui.

Aos animais utilizados nesta pesquisa, expresso minha mais profunda gratidão e respeito. Reconheço que o avanço da ciência, muitas vezes, depende de suas contribuições silenciosas e involuntárias. Sem eles, não existiriam avanços e sem avanços, não existiríamos. Que este trabalho honre suas vidas e reforce o compromisso com o uso ético, responsável e racional de seres destinados à pesquisa.

À Agroceres Multimix Nutrição Animal, especialmente aos colaboradores Me. Tarley Araújo Barros e Drª. Nayla Pádua Del Bianco Gontijo Souki, agradeço o apoio e suporte oferecidos para a execução deste trabalho.

Agradeço, ainda, o fomento e o incentivo à pesquisa concedidos pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), processo nº 2024/03279-0 e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), código de financiamento 001.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001

SUMÁRIO

Página

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Avaliação da adição de óleos essenciais na dieta de frangos de corte visando o controle da colonização intestinal por *Salmonella Enteritidis*"**, protocolo nº 2931/2024, sob a responsabilidade do Prof. Dr. ANGELO BERCHIERI JUNIOR, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 26 de junho de 2024.

(We certify that the research project entitled **"Evaluation of the essential oils added into poultry feed aiming to control enteric colonization by *Salmonella Enteritidis*"**, protocol nº. 2931/2024, under the responsibility of Dr. ANGELO BERCHIERI JUNIOR, which involves the production, maintenance and/or use of animals belonging to the Chordata phylum, vertebrate subfile (except man), for the purposes of scientific research (or teaching) - is in accordance with the precepts of Law no. 11,794, of October 8, 2008, in Decree 6,899, of July 15, 2009, and with the rules issued by the National Council for Animal Experimentation Control (CONCEA), and was approved by the Ethics Commission on Animal Use (CEUA), from the SCHOOL OF AGRICULTURAL AND VETERINARY SCIENCES, UNESP - JABOTICABAL - SP Campus, at June, 26, 2024 an ordinary meeting.)

Vigência do Projeto	01/10/2024 a 01/02/2025
Espécie / Linhagem	Frangos de corte
Nº de animais	200
Peso / Idade	1 dia de idade
Sexo	Machos e Fêmeas
Origem	Incubatório Comercial

Jaboticabal, 26 de junho de 2024

Helena C D Brito

Dra. Helena Cristina Delgado Brito
Coordenadora – CEUA

AVALIAÇÃO DA ADIÇÃO DE ÓLEOS ESSENCIAIS NA DIETA DE FRANGOS DE CORTE VISANDO O CONTROLE DA COLONIZAÇÃO INTESTINAL POR *Salmonella* Enteritidis

RESUMO – *Salmonella* Enteritidis (SE) é um importante patógeno para a avicultura e a saúde pública. Diante das restrições ao uso de antimicrobianos, os óleos essenciais (OEs) têm sido explorados como alternativas naturais devido às suas propriedades antimicrobianas. Assim, este estudo avaliou o efeito de OEs adicionados à dieta de frangos de corte experimentalmente infectados com SE. Inicialmente, 16 OEs foram testados *in vitro* por meio da CIM, CBM e CIF, sendo que canela, orégano, tomilho-branco, cravo e menta apresentaram os melhores resultados. O *blend* selecionado (canela + orégano + cravo) foi microencapsulado e posteriormente avaliado *in vivo*. No experimento, 135 pintinhos de um dia de vida foram infectados com SE e distribuídos em três grupos: dois tratados com o *blend* microencapsulado em diferentes dosagens e um grupo controle positivo. A excreção fecal de SE foi monitorada por suabes cloacais realizados duas vezes por semana, e nos dias 2, 5, 7, 14 e 21 pós-infecção cinco aves de cada grupo foram sacrificadas para contagem bacteriana em conteúdo cecal, fígado e baço. O consumo diário de ração também foi registrado para verificar possíveis efeitos sobre a palatabilidade. A suplementação com metade da dose do *blend* resultou em redução de 36 % na excreção fecal e diminuição de 4 log_{10} na colonização cecal, além de redução transitória na colonização hepática, sem alterar o consumo de ração. Esses resultados indicam que OEs microencapsulados podem servir como uma estratégia natural e eficaz para o controle de SE em frangos de corte. Estudos adicionais sobre otimização de doses, impacto na microbiota intestinal, segurança e desempenho produtivo são essenciais para sua futura aplicação comercial.

Palavras-chave: Antimicrobianos, fitobióticos, salmoneloses, saúde única.

EVALUATION OF THE ESSENTIAL OILS ADDED INTO POULTRY FEED AIMING TO CONTROL ENTERIC COLONIZATION BY *Salmonella* Enteritidis

ABSTRACT – *Salmonella* Enteritidis (SE) is an important pathogen for poultry production and public health. Due to restrictions on the use of antimicrobials, essential oils (EOs) have been explored as natural alternatives because of their antimicrobial properties. Therefore, this study evaluated the effect of dietary EOs in broiler chickens experimentally infected with SE. Initially, 16 EOs were tested in vitro using MIC, MBC, and FIC assays, and cinnamon, oregano, white thyme, clove, and mint showed the strongest antimicrobial activity. The selected blend (cinnamon + oregano + clove) was microencapsulated and subsequently evaluated in vivo. In the animal trial, 135 one-day-old chicks were infected with SE and assigned to three groups: two treated with different doses of the microencapsulated blend and one positive control group. Fecal shedding of SE was monitored twice weekly through cloacal swabs, and on days 2, 5, 7, 14, and 21 post-infection, five birds from each group were euthanized to quantify SE in cecal content, liver, and spleen. Daily feed intake was also recorded to assess potential effects on palatability. Supplementation with half the optimized dose resulted in a 36 % reduction in fecal shedding and a 4 $\log_{10}$ decrease in cecal colonization, along with a transient reduction in hepatic colonization, without affecting feed consumption. These findings indicate that microencapsulated EOs may serve as a natural and effective strategy for controlling SE in broilers. Further studies on dose optimization, impacts on intestinal microbiota, safety, and productive performance are essential for future commercial applications.

Keywords: Antimicrobials, One Health, phytobiotics, salmonellosis.

CAPÍTULO 1 – Considerações gerais

1. Introdução

A infecção de aves por salmonelas paratíficas, como *Salmonella* Enteritidis (SE), frequentemente se manifesta de forma assintomática em planteis comerciais, sendo disseminada entre as aves, no ambiente e nos produtos avícolas (Guard-Petter, 2001). Isso dificulta a detecção precoce e aumenta os riscos de contaminação cruzada para humanos, sendo os sorovares *S.* Enteritidis e *S.* Typhimurium os mais prevalentes em infecções alimentares oriundas de produtos avícolas (Karabasanavar et al., 2020). A cada ano, as perdas econômicas associadas a salmonelas avícolas, como despesas hospitalares e embargos comerciais, podem ultrapassar os US$ 2,8 bilhões para a economia dos Estados Unidos da América (Scharff, 2020). Portanto, várias estratégias têm sido propostas para mitigar a ocorrência de *S. enterica* em aves de produção.

Neste contexto, estratégias que visem reduzir a carga bacteriana antes do abate são consenso como um dos principais métodos para prevenir a disseminação de *Salmonella* spp. Aljuwayd et al., (2023) destacam o uso de fitobióticos na ração como alternativa no combate de microrganismos indesejáveis. Os fitobióticos — que incluem óleos essenciais (OEs), extratos vegetais, ervas e especiarias medicinais — são produtos provenientes de plantas e são pesquisados devido ao seu efeito antimicrobiano natural para aves e outras espécies (Suresh et al., 2018).

Além de ser uma alternativa natural, os OEs não provocam resistência à antibióticos e são efetivos mesmo sobre estirpes multirresistentes (Alibi et al., 2022). Isso se deve a variedade de moléculas antimicrobianas que lhes compõe e por possuírem múltiplos alvos na célula bacteriana (Suresh et al., 2018). Além disso, os OEs podem atuar sinergicamente com outros óleos, o que permite reduzir a concentração de um ou ambos e otimizar o seu efeito antimicrobiano contra *Salmonella* spp. (Rodrigues et al., 2025).

Por outro lado, algumas limitações ainda são observadas em terapias fitobióticas em aves, incluindo a alta volatilidade dos compostos, ausência de padronização de doses terapêuticas e tóxicas, bem como seus possíveis efeitos sobre a microbiota e a integridade intestinal (Aljuwayd et al., 2023; Rodrigues et al., 2025; Suresh et al., 2018). Segundo Ibrahim et al. (2021), a suplementação de OEs na dieta de frangos de corte pode modular a expressão de genes associados à barreira intestinal, como *MUC-2* e

FABP2, os quais estão diretamente relacionados à manutenção da integridade da mucosa e à proteção contra a invasão de patógenos entéricos. Nesse sentido, embora promissores, os efeitos regulatórios dos OEs sobre o organismo da ave — especialmente na integridade intestinal — e seu papel no controle de *Salmonella enterica* em frangos de corte ainda carecem de maior elucidação, reforçando a necessidade de investigações adicionais.

2. Objetivos

2.1. Objetivo geral

O objetivo deste trabalho foi de avaliar o efeito antimicrobiano de OEs adicionados à ração de frangos de corte visando controlar a colonização intestinal por SE.

2.2. Objetivos específicos

I. Avaliar a Concentração Inibitória Mínima (CIM), Concentração Bactericida Mínima (CBM) e Concentração Inibitória Fracionada (CIF) de dezesseis OEs;

II. Confeccionar um *blend* de OEs microencapsulados a partir dos óleos com os melhores resultados nos ensaios *in vitro*;

III. Avaliar o efeito antimicrobiano do *blend* de OEs quanto à colonização cecal e excreção fecal de SE em frangos de corte;

IV. Avaliar o consumo diário de ração em frangos tratados com e sem o *blend* de OEs.

3. Revisão de Literatura

3.1. *Salmonella enterica* subsp. *enterica* sorovar Enteritidis

SE pertence à família Enterobacteriaceae e consiste em bastonetes Gram-negativos, anaeróbios facultativos (Freitas Neto et al., 2020). A transmissão pode ocorrer de forma horizontal e vertical, favorecendo sua rápida disseminação nos aviários e dificultando o controle (Berchieri Jr et al., 2001; Oliveira et al., 2000; Zancan et al., 2000). Quando se trata de uma salmonelose aviária não causada pelos sorovares *S.* Gallinarum e *S.* Pullorum, ela é denominada de infecção paratífica (Oliveira et al., 2000). Sendo SE e *S*. Typhimurium os sorotipos mais frequentes em infecção alimentares humanas oriundas de produtos avícolas (Freitas Neto et al., 2020).

A presença de *Salmonella* spp. em produtos avícolas é motivo de preocupação para as autoridades sanitárias internacionais, pois impede a comercialização desses produtos devido ao risco de infecção em humanos e outras espécies (Abou-Jaoudeh et al., 2024). Historicamente, os casos de salmonelose em humanos aumentaram consideravelmente a partir da década de 1970, sendo o sorovar SE um dos mais frequentes a partir dos anos 1980, principalmente em produtos avícolas (Guard-Petter, 2001). Para reduzir o risco de infecção alimentar em humanos, diversos países implementaram programas de prevenção e controle de *Salmonella* spp. em aves de produção (Guard-Petter, 2001; Freitas Neto et al., 2020; Saraiva et al., 2022).

Em humanos, o quadro clínico geralmente é caracterizado por intensa diarreia, dor abdominal, febre e episódios de êmese. No entanto, em indivíduos imunocomprometidos, idosos ou crianças, a infecção pode ser fatal (Sodagari et al., 2020). Em plantéis avícolas, a infecção por SE pode se manifestar de forma aguda ou crônica (Enan et al., 2023; Moharreri et al., 2022). Aves jovens são mais susceptíveis à infecção, o que pode dificultar o diagnóstico diferencial com a pulorose (Freitas Neto et al., 2020).

A infecção por SE pode levar a um desequilíbrio da microbiota intestinal e a colonização de órgãos como baço, coração, ovário e fígado (Enan et al., 2023; Hu et al., 2023). Aves infectadas podem apresentar diarreia, atraso no crescimento e aumento das taxas de morbidade e mortalidade (Hu et al., 2023). Contudo, é frequente o isolamento de estirpes de SE em aves assintomáticas (Freitas Neto et al., 2020).

A epidemiologia das salmonelas paratíficas é complexa, pois a bactéria pode estar presente em diversos ambientes e hospedeiros, incluindo aviários, incubatórios, aves, ovos, aves silvestres, roedores e humanos (Guard-Petter, 2001). Sua persistência em granjas está relacionada à capacidade de colonizar os tratos digestivo e reprodutivo das aves, além de sua disseminação por fômites contaminados (Berchieri Jr et al., 2001; Ferreira et al., 2022; Zancan et al., 2000). Ademais, aves infectadas precocemente podem excretar a bactéria por longos períodos, contribuindo para sua manutenção e disseminação no ambiente (Berchieri Jr et al., 2001).

3.2. Resistência aos antimicrobianos e impacto econômico

Um dos principais problemas observados no tratamento das salmoneloses humanas e de outras infecções bacterianas é o aumento da resistência das bactérias aos antimicrobianos, especialmente em decorrência do uso indiscriminado desses fármacos na produção animal (Sodagari et al., 2020). Embora esta resistência seja um fenômeno natural e amplamente distribuído entre as bactérias (D'Costa et al., 2011), fatores antropogênicos têm contribuído para a intensificação desse problema (Saraiva et al., 2022).

Na avicultura, em particular, os antimicrobianos são amplamente empregados não apenas para a prevenção e o tratamento de enfermidades bacterianas, mas também como melhoradores de desempenho, em doses subterapêuticas (Thompson et al., 2023). Como consequência, estirpes resistentes ou multirresistentes de *Salmonella enterica* e de outras bactérias patogênicas têm sido frequentemente isoladas de produtos avícolas (Abou-Jaoudeh et al., 2024).

A Organização Mundial da Saúde (OMS) reconheceu, em 2023, a resistência aos antimicrobianos como uma das principais ameaças à saúde pública global. Estima-se que, até 2050, essa resistência seja responsável por mais de 10 milhões de óbitos anuais e por um prejuízo econômico próximo de 100 trilhões de dólares (OMS, 2023). Além dos custos hospitalares decorrentes do tratamento de salmoneloses paratíficas multirresistentes, perdas adicionais relacionadas a embargos comerciais, mortalidade e redução da produtividade podem ultrapassar 2,8 bilhões de dólares por ano (Scharff, 2020).

3.3. Alternativas para a prevenção de salmoneloses de interesse na avicultura e saúde pública

Em decorrência do agravamento dos problemas médico-hospitalares e econômicos associados à rápida disseminação de patógenos resistentes a antimicrobianos, legislações que restringem e racionalizam o uso dessas drogas em animais de produção têm incentivado a busca por novas alternativas com atividade antimicrobiana e sem efeitos adversos à saúde animal e humana (Bona et al., 2012; Gadde et al., 2017; Saraiva et al., 2022). Entre as principais alternativas estudadas estão os prebióticos, probióticos, ácidos orgânicos e OEs, devido à sua capacidade de favorecer o equilíbrio da microbiota intestinal e inibir a multiplicação de microrganismos indesejáveis no trato entérico das aves (Bona et al., 2012; Meenu et al., 2023; Murate et al., 2015; Stingelin et al., 2023).

Os prebióticos atuam como substratos seletivos para determinados microrganismos benéficos que compõem a microbiota intestinal das aves, enquanto os probióticos consistem em microrganismos viáveis capazes de colonizar e exercer efeitos benéficos em compartimentos específicos do hospedeiro (Murate et al., 2015; Schrezenmeir e Vrese, 2001).

Os ácidos orgânicos, por sua vez, são definidos como compostos que contêm uma ou mais carboxilas em sua estrutura molecular. Podem ser saturados ou insaturados e pertencem a um amplo grupo de ácidos carboxílicos com cadeias de hidrocarbonetos alifáticos (Milbradt e Andreatti Filho, 2020). Sua ação antimicrobiana está relacionada à capacidade de alterar o pH intracelular bacteriano e de interagir diretamente com a parede celular de bactérias Gram-negativas (Dittoe et al., 2018; Immerseel et al., 2007).

Os OEs são metabólitos secundários produzidos por diversas partes das plantas — como flores, folhas, raízes e caules — e apresentam uma grande diversidade de princípios ativos que desempenham papel fundamental nos mecanismos de defesa vegetal, especialmente devido à sua ação antimicrobiana (Meenu et al., 2023). Em função disto, estes fitobióticos são propostos como alternativa para substituírem o uso de antimicrobianos sintéticos na produção animal (Bernardos et al., 2014; Bona et al., 2012; Penteado et al., 2021).

3.4. Atividade antimicrobiana dos OEs

Os OEs são extraídos de plantas medicinais, condimentares e aromáticas, possuindo ação contra bactérias Gram-positivas, Gram-negativas e contra fungos (filamentosos e leveduras) (Bernardos et al., 2014; Penteado et al., 2021; Oliveira Filho et al., 2023). Além do uso consolidado na indústria de alimentos, os OEs também vêm sendo incorporados à dieta de aves com o objetivo de melhorar o desempenho produtivo (Bona et al., 2012; Moharreri et al., 2022; Penteado et al., 2021). Estudos recentes também demonstraram seu potencial na eliminação de contaminantes bacterianos presentes na cama de aviários (Galgano et al., 2023).

Embora haja evidências científicas da atividade antimicrobiana desses compostos, as pesquisas voltadas ao uso de OEs para o controle de infecções bacterianas em animais ainda são limitadas (Nuñez et al., 2024). Alguns estudos apontam que os OE de cebola, alho, copaíba, gengibre e hortelã podem ser eficazes no controle *in vitro* sobre *Salmonella* spp. (Iwinski et al., 2022; Somrani et al., 2023). Assim, por serem substâncias seguras ao consumo e apresentarem efeito antimicrobiano comprovado *in vitro*, acredita-se que os OEs possam atuar de forma eficaz na redução da excreção de *Salmonella* spp. em aves de produção (Rodrigues et al., 2025).

O mecanismo de ação dos OEs varia conforme o composto ativo predominante. De modo geral, o potencial antimicrobiano está associado na ruptura da parede celular bacteriana, desnaturação de proteínas e aumento da permeabilidade da membrana a cátions, como H^+ e K^+ (Bona et al., 2012). Tanto o mecanismo quanto a eficácia antimicrobiana variam de acordo com o tipo de patógeno envolvido (Nuñez et al., 2024). Para otimizá-la, a combinação de um ou mais OEs pode resultar em uma melhora da eficácia antimicrobiana do que quando utilizados individualmente (Meenu et al., 2023).

O carvacrol é um dos principais constituintes dos OEs extraídos de plantas aromáticas, como o orégano (*Origanum vulgare*) e a manjerona (*Origanum marjorana*) (Bona et al., 2012; Silva et al., 2012). Essa substância, assim como outros compostos presentes nos OEs, é reconhecida como segura para uso medicinal pela *Food and Drug Administration* (FDA) (Oliveira Filho et al., 2023; Silva et al., 2012). Avaliações *in vitro* demonstraram que o carvacrol apresenta forte atividade antibacteriana, especialmente contra *Escherichia coli* e *S.* Typhimurium (Severino et al., 2015). De acordo com Bona et al. (2012), a associação de carvacrol com outros OEs reduziu a excreção fecal de SE, *Clostridium perfringens* e *Eimeria maxima* em frangos de corte experimentalmente infectados.

O timol é outro princípio ativo de destaque, presente em OEs de plantas como o tomilho (*Thymus vulgaris*) (Meenu et al., 2023). Trata-se de um fenol pertencente ao grupo dos monoterpenos, amplamente utilizado na indústria alimentícia como conservante natural (Cabral et al., 2009). Sua ação antibacteriana sobre *Listeria monocytogenes* e diversos sorovares de *Salmonella* spp. já foi observada *in vitro* e *in vivo* (Bona et al., 2012; Meenu et al., 2023). Além de apresentar segurança quanto à ingestão, o timol também é utilizado por suas propriedades antioxidantes e como componente de formulações repelentes (Cabral et al., 2009).

O eugenol, pertencente ao grupo dos fenilpropenos, assim como o cinamaldeído, apresenta ampla atividade antibacteriana, sendo especialmente eficaz contra bactérias Gram-negativas (Meenu et al., 2023). Esses compostos estão presentes nos OEs de cravo (*Syzygium aromaticum*) e canela (*Cinnamomum verum*), e, além da ação antibacteriana, também exibem propriedades antifúngicas, sendo amplamente empregados como antimicrobianos naturais (Oliveira Filho et al., 2023).

Apesar do reconhecido potencial biológico, algumas limitações restringem o uso direto dos OEs, como a baixa solubilidade em água, alta volatilidade, suscetibilidade à degradação pela luz e possível toxicidade em altas concentrações (Oliveira Filho et al., 2023). Entretanto, o encapsulamento desses óleos tem se mostrado uma alternativa promissora para superar tais limitações, conferindo maior estabilidade e proteção, além de permitir um controle mais eficiente da liberação de seus componentes ativos (Liu et al., 2019).

CAPÍTULO 2 – Essential oils used in the poultry industry: Would it be an effective green alternative against *Salmonella* spp. dissemination and antimicrobial resistance?[1]

Heitor Leocádio de Souza Rodrigues[1], Isis Mari Miyashiro Kolososki[1], Valdinete Pereira Benevides[1], Mauro M. S. Saraiva[1*], Angelo Berchieri Junior[1*].

[1]São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, Brazil. *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. mauro.saraiva@unesp.br and angelo.berchieri@unesp.br.

Highlights

- Essential oils exhibit *in vitro* and *in vivo* antimicrobial activity against *Salmonella* spp.

[1] Este capítulo corresponde ao artigo científico publicado na revista *The Microbe* em 2025.

- The antibacterial mechanism of essential oils can be involved in damage to the bacterial cell wall.
- Essential oils can enhance broiler performance and reduce *Salmonella enterica* excretion.
- Encapsulation of essential oils improves the bioavailability of the phytobiotic in poultry.
- Studies reaching the ideal concentration of Essential oils remain a gap in that field.

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.

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

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*) species, 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, Zhang et al., 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 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).

EOs, 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

EOs 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 Ɣ-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 EO 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 Figure 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.

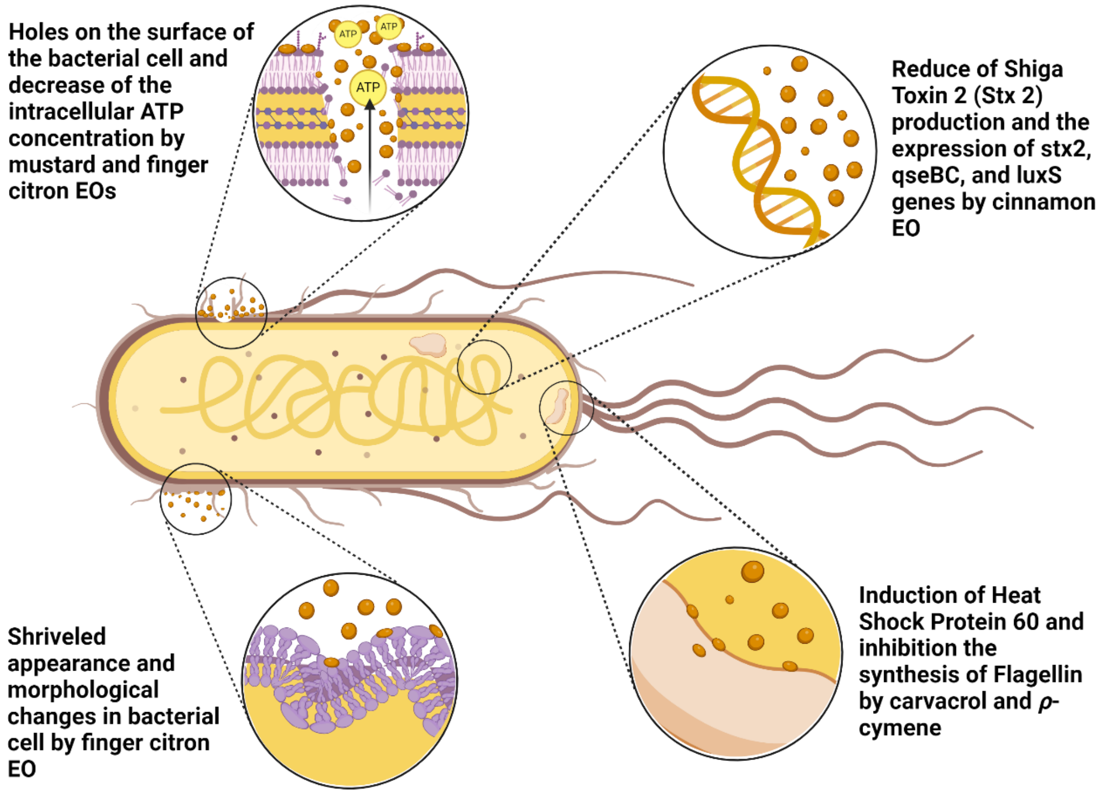


Figure 1: Main alterations reported in bacterial cells due to contact with essential oils and their chemical components, according to Burt et al. (2007); Frazzon et al. (2018); Li et al. (2019); Turgis et al. (2009). This figure was created using BioRender.com.

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 EO 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 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).

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).

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 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 EO 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 EOs 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 (Figure 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).

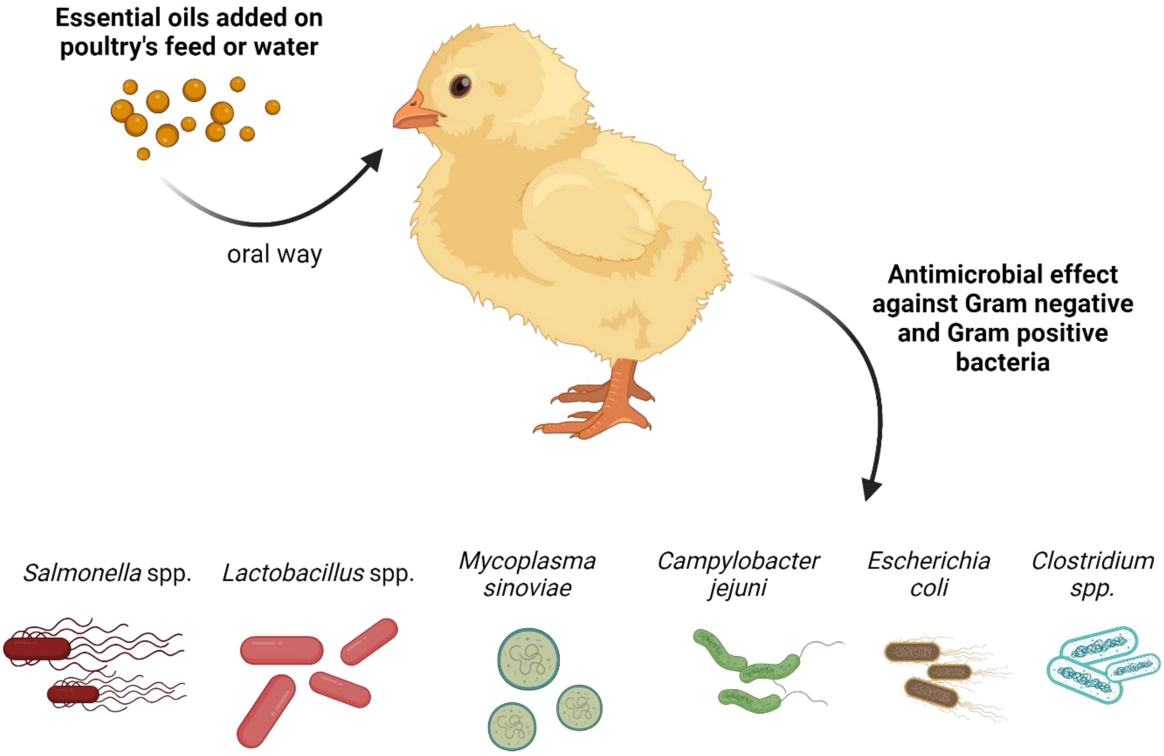


Figure 2: Antimicrobial effect of EOs added on poultry's feed or water against Gram-positive and Gram-negative bacteria. This figure was created using BioRender.com.

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 with other dietary lipids and are metabolized in the liver after a first-pass effect (Horky et al., 2019), as illustrated in Figure 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 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).

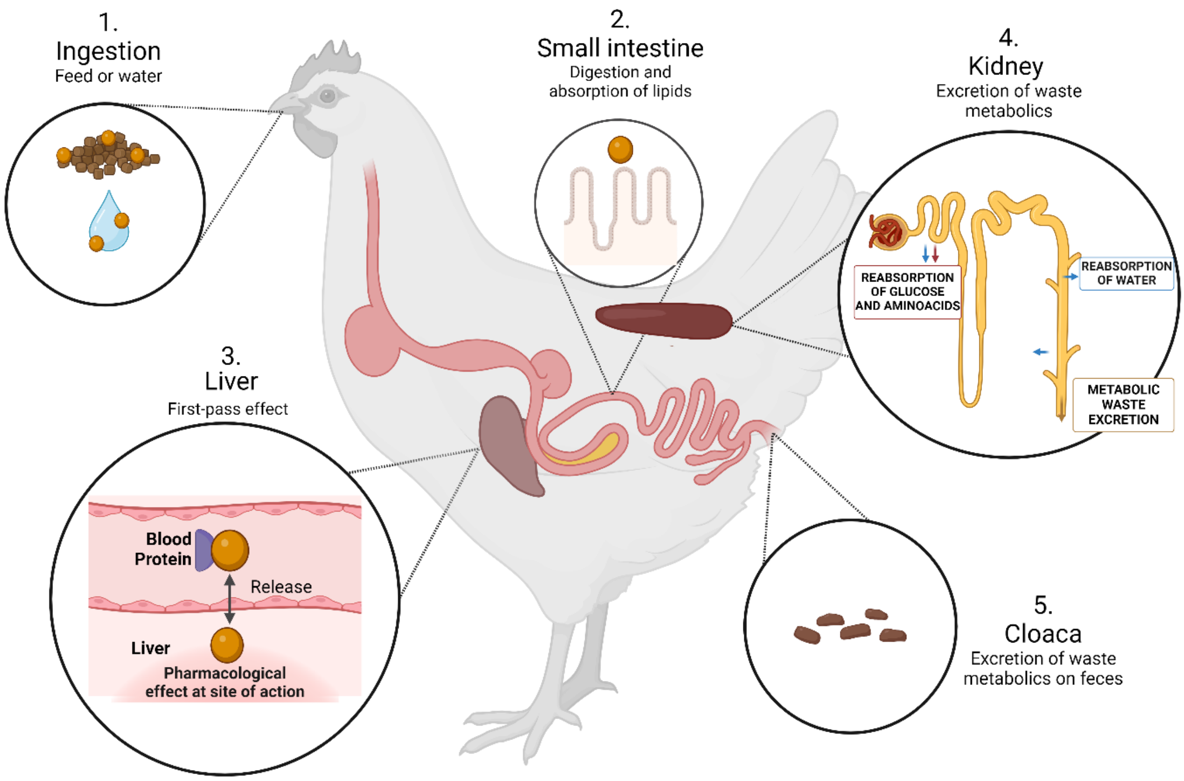


Figure 3: Probable pharmacokinetic pathways of essential oils in the chicken organism, according to Attia et al. (2019); Gadde et al. (2017); Horky et al. (2019); Puvaca et al. (2020). This figure was created using BioRender.com.

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 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., 2021, Zhang et al., 2021). 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 contribuition 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 Fundação de Amparo à Pesquisa do Estado de São Paulo (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.

References

Abers, M., Schroeder, S., Goelz, L., Sulser, A., Rose, T.S., Puchalski, K., Langland, J., 2021. Antimicrobial activity of volatile substances from essential oils. BMC Complement Med. Ther. 21 (124). https://doi.org/10.1186/s12906-021-03285-3.

Abramovici, A., Rachmuth-Raizman, P., 1983. Molecular structure-teratogenicity relationships of some fragrance additives. Toxicology 29 (1-2), 143–156. https://doi.org/10.1016/0300-483X(83)90046-X.

Ali, R.A., Elzahraa, D., Mostafa, F., Aboulqasem, H.E., 2019. The effect of co-treatment with retinoic acid on rescuing citral induced morphological anomalies during chick embryo development. Assiut Univ. J. Zool. 1 (1), 42–63.

Andrade, B.F., Ferreira, V.R.F., Cardoso, G.P., et al., 2023. Allspice (Pimenta dioica Lindl.) leaves essential oils as a potential antioxidant and antimicrobial source for use in mechanically deboned poultry meat. Braz. J. Food Technol. 26, 2022125. https://doi.org/10.1590/1981-6723.12522.

Attia, Y., Al-Harthi, M., El-Kelawy, M., 2019. Utilization of essential oils as a natural growth promoter for broiler chickens. Ital. Journ. Anim. Sci. 18 (1), 1005–1012. https://doi.org/10.1080/1828051X.2019.1607574.

Azevedo, I.L., Nogueira, W.C.L., Almeida, A.C., Guedes, L.L.M., Vieira, C.R., Santos, S.H. S., Carvalho, C.M.C., Fonseca, F.S.A., Souza, R.M., Souza, C.N., 2021. Antioxidant activity and chemical composition of meat from broilers feed diets containing different essential oils. Vet. World 14 (6), 1638–1643. https://doi.org/10.14202/vetworld.2021.1638-1643.

Aziz, Z.A.A., Ahmad, A., Setapar, S.H.M., Karakucuk, A., Azim, M.M., Lokhat, D., et al., 2018. Essential oils: extraction techniques, pharmaceutical and therapeutic potential – a review. Curr. Drug Metab. 19 (13), 1100–1110. https://doi.org/10.2174/1389200219666180723144850.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils – a review. Food Chem. Tox 46 (2), 446–475. https://doi.org/10.1016/j.fct.2007.09.106.

Baptiste, K.E., Pokludova, L., 2020. Mass medications: prophylaxis and metaphylaxis, cascade and off-label use, treatment guidelines and antimicrobial stewardship. Springer. https://doi.org/10.1007/978-3-030-46721-0_7.

Barbosa, L.N., Alves, F.C.B., Andrade, B.F.M.T., Albano, M., Rall, V.L.M., Fernandes, A.A. H., Buzalaf, M.A.R., Leite, A.L., Pontes, L.G., Santos, L.D., Fernandes Junior, A., 2020. Proteomic analysis and antibacterial resistance mechanisms of *Salmonella* Enteritidis submitted to the inhibitory effect of Origanum vulgare essential oil, thymol and carvacrol. J. Proteom. 214 (1). https://doi.org/10.1016/j.jprot.2019.103625.

Barrow, P.A., Berchieri Junior, A., Freitas Neto, O.C., Lovell, M., 2015. The contribution of aerobic and anaerobic respiration to intestinal colonization and virulence for *Salmonella* Typhimurium in the chicken. Avian Pathol. 44 (5), 401–407. https://doi.org/10.1080/03079457.2015.1062841.

Bartkiene, E., Ruzauskas, M., Bartkevics, V., Pugajeva, I., et al., 2020. Study of the antibiotic residues in poultry meat in some of the EU countries and selection of the best composition of lactic acid bacteria and essential oils against *Salmonella enterica*. Poult. Sci. 99 (8), 4065–4076. https://doi.org/10.1016/j.psj.2020.05.002.

Beier, R.C., Byrd, J.A., Kubena, L.F., Hume, M.E., McReynolds, J.L., Anderson, R.C., Nisbet, D.J., 2014. Evaluation of linalool, a natural antimicrobial and insecticidal essential oil from basil: effects on poultry. Poult. Sci. 93 (2), 267–272. https://doi. org/10.3382/ps.2013-03254.

Benevides, V.P., Saraiva, M.M.S., Nascimento, C.F., Delgado-Suar´ez, E.J., Oliveira, C.J.B., Silva, S.R., Miranda, V.F.O., Christensen, H., Olsen, J.E., Berchieri Junior, A., 2024. Genomic features and phylogenetic analysis of antimicrobial-resistant *Salmonella* Mbandaka ST413 strains. Microorganisms 12 (2), 312. https://doi.org/10.3390/microorganisms12020312.

Bona, T.D.M.M., Pickler, L., Miglino, L.B., et al., 2012. Óleo essencial de orégano, alecrim, canela e extrato de pimenta no controle de *Salmonella*, *Eimeria* e *Clostridium* em frangos de corte. Pesq. Vet. Bras. 32 (5). https://doi.org/10.1590/S0100-736X2012000500009.

Boy, F.R., Benito, M.J., Cordoba, ´ M.G., et al., 2023. Antimicrobial properties of essential oils obtained from autochthonous aromatics plants. Int. J. Environ. Res. Public Health 20 (3), 1657. https://doi.org/10.3390/ijerph20031657.

Burt, S.A., Zee, R.V.D., Koets, A.P., Graaff, A.M., et al., 2007. Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in *Escherichia coli* O157:H7. Appl. Environ. Microb. 73 (14), 4484–4490. https://doi.org/10.1128/AEM.00340-07.

Cabarkapa, I., Puvaca, N., Popovic, S., Colovic, D., Kostadinovic, L., Tatham, E.K., Levic, J., 2020. Aromatic plants and their extracts pharmacokinetics and *in vitro/in vivo* mechanisms of action. Feed Addit. 75–88. https://doi.org/10.1016/B978-0-12- 814700-9.00005-4.

Caneschi, A., Bardhi, A., Barbarossa, A., Zaghini, A., 2023. The use of antibiotics and antimicrobial resistance in veterinary medicine, a complex phenomenon: a narrative review. Antibiotics 12 (3), 487. https://doi.org/10.3390/antibiotics12030487.

Cariri, M.L., Melo, A.N.F., Mizzi, L., et al., 2019. Quantitative assessment of tolerance response to stress after exposure to oregano and rosemary essential oils, carvacrol and 1,8-cineole in *Salmonella* Enteritidis 86 and its isogenic deletion mutants Δ*dps*, Δ*rpoS* and Δ*ompR*. Food Res. Int. 122 (1), 679–687. https://doi.org/10.1016/j. foodres.2019.01.046.

Centers for Disease Control and Prevention (CDC). 2018. *Salmonella* Typhimurium linked to chicken salad (final update). Available in: https://archive.cdc.gov/www_cdc_gov/salmonella/typhimurium-02-18/index.html.

Chlebicz, A., Slizewska, K., 2018. Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. Int. J. Environ. Res. Public Health. 15 (5), 863. https://doi.org/10.3390/ijerph15050863.

Cimino, C., Maurel, O.M., Musumeci, T., et al., 2021. Essential oils: pharmaceutical applications and encapsulation strategies into lipid-based delivery systems. Pharmaceutics 13, 327. https://doi.org/10.3390/pharmaceutics13030327.

Coles, M.E., Forga, A.J., Senas-Cuesta, ˜ R., et al., 2021. Assessment of Lippia origanoides essential oils in a *Salmonella* Typhimurium, *Eimeria maxima*, and *Clostridium perfringens* challenge model to induce necrotic enteritis in broiler chickens. Animals. 11 (4), 1111. https://doi.org/10.3390/ani11041111.

Di Vito, M., Cacaci, M., Barbanti, L., et al., 2020. Origanum vulgare essential oil vs. commercial mixture of essential oils: In vitro effectiveness on *Salmonella* spp. from poultry and swine intensive livestock. Antibiotics 9 (11), 763. https://doi.org/ 10.3390/antibiotics9110763.

Diaz-Sanchez, S., D’Souza, D., Biswas, D., Hanning, I., 2015. Botanical alternatives to antibiotics for use in organic poultry production. Poult. Sci. 94 (6), 1419–1430. https://doi.org/10.3382/ps/pev014.

Ebani, V.V., Najar, B., Bertelloni, F., Pistelli, L., Mancianti, F., Nardoni, S., 2018. Chemical composition and *in vitro* antimicrobial efficacy of sixteen essential oils against *Escherichia coli* and *Aspergillus fumigatus* isolated from poultry. Vet. Sci. 5 (3), 62. https://doi.org/10.3390/vetsci5030062.

Ebani, V.V., Nardoni, S., Bertelloni, F., Tosi, G., Massi, P., Pistelli, L., Mancianti, F., 2019. *In vitro* antimicrobial activity of essential oils against *Salmonella enterica* serotypes Enteritidis and Typhimurium strains isolated from poultry. Molecules 24 (5). https://doi.org/10.3390/molecules24050900.

EFSA, Bampidis, V., Azimonti, G., Bastos, M.L., et al., 2019. Safety and efficacy of an essential oil from Origanum vulgare ssp. hirtum (Link) Ietsw. for all animal species. EFSA J. https://doi.org/10.2903/j.efsa.2019.5909.

El-Dayem, G.A.A., Shalaby, M., Elkenawy, M.E., 2024. Effects of some essential oils on growth performance and *Campylobacter jejuni* in broilers. J. Adv. Vet. Res. 14 (3), 384–389. https://advetresearch.com/index.php/AVR/article/view/1589.
El-Hack, M.E.A., El-Saadony, M.T., Salem, H.M., et al., 2022. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. Poult. Sci. 101. https://doi.org/10.1016/j.psj.2022.101696.
El-Shall, N.A., Shewita, R.S., El-Hack, M.E.A., et al., 2020. effect of essential oils on the immune response to some viral vaccines in broiler chickens, with special reference to Newcastle disease virus. Poult. Sci. 99 (6), 2944–2954. https://doi.org/10.1016/j.psj.2020.03.008.
European Centre for Disease Prevention and Control (ECDPC) and European Food Safety Authority (EFSA). 2022. Multi-country outbreak of *Salmonella* Mbandaka ST413, possibly linked to consumption of chicken meat in the EU/EEA, Israel and the UK. Available in: https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2022. EN-7707.
European Union (EU). Regulation (EU) on the manufacture, placing on the market and use of medicated feed, amending Regulation (EC) No 183/2005 of the European Parliament and of the Council and repealing Council Directive 90/167/EEC. Official Journal of the European Union. 2019. Available in: https://eur-lex.europa.eu/legal - content/EN/TXT/PDF/?uri=CELEX:32019R0004&from=EN.
Evangelista, A.G., Corrˆea, J.A.F., Pinto, A.C.S.M., Luciano, F.B., 2021. The impact of essential oils on antibiotic use in animal production regarding antimicrobial resistance – a review. Crit. Rev. Food Sci. Nutr. 62 (1), 5267–5283. https://doi.org/10.1080/10408398.2021.1883548.
Ferrentino, G., Morozova, K., Horn, C., Scampicchio, M., 2020. Extraction of essential oils from medicinal plants and their utilization as food antioxidants. Curr. Pharm. Des. 26 (1), 519–541. https://doi.org/10.2174/1381612826666200121092018.
Frazzon, A.P.G., Saldanha, M., Lauer Junior, C., Frazzon, J., 2018. Modulation of gene expression by essential oils in bacteria. Adv. Biot. Microb. 8 (2), 555731. https://doi.org/10.19080/AIBM.2018.08.555731.
Gadde, U., Kim, W.H., Oh, S.T., et al., 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev.

18 (1), 26–45. https://doi.org/10.1017/s1466252316000207.
Galgano, M., Pellegrini, F., Fracchiolla, G., et al., 2023. Pilot study on the action of Thymus vulgaris essential oil in treating the most common bacterial contaminants and *Salmonella enterica* subsp. *enterica* serovar Derby in poultry litter. Antibiotics 12 (3), 436. https://doi.org/10.3390/antibiotics12030436.
Gharaibeh, M.H., Khalifeh, M.S., Nawasreh, A.N., et al., 2021. Assessment of immune response and efficacy of essential oils applied on controlling necrotic enteritis induced by *Clostridium perfringens* in broiler chickens. Molecules 26 (15), 4527. https://doi.org/10.3390/molecules26154527.
Gieraltowski, L., Peralta, J.H.V., Green, A., Schwensohn, C., Rosen, H., et al., 2016. National outbreak of multidrug resistant *Salmonella* Heidelberg infections linked to a single poultry company. PLOS One. https://doi.org/10.1371/journal.pone.0162369.
Girard, C., Chabrillat, T., Kerros, S., Fravalo, P., Thibodeau, A., 2024. Essential oils mix effect on chicks ileal and caecal microbiota modulation: a metagenomics sequencing approach. Front. Vet. Sci. 11 (4). https://doi.org/10.3389/fvets.2024.1350151.Hoffmann, K.H., 2020. Essential oils. Z. Naturforsch. 75 (1), 7–8. https://doi.org/10.1515/znc-2020-0124.
Horky, P., Skalickova, S., Smerkova, K., Skladanka, J., 2019. Essential oils as a feed additives: pharmacokinetics and potential toxicity in monogastric animals. Animals. 9 (6), 352. https://doi.org/10.3390/ani9060352.
Hu, Z., Liu, L., Guo, F., et al., 2023. Dietary supplemental coated essential oils and organic acids mixture improves growth performance and gut health along with reduces *Salmonella* load of broiler chickens infected with *Salmonella* Enteritidis. J. Anim. Sci. Biotechnol. 14 (95). https://doi.org/10.1186/s40104-023-00889-2.
Huang, J., Guo, F., Abbas, W., 2024. Effects of microencapsulated essential oils and organic acids preparations on growth performance, slaughter characteristics, nutrient digestibility, and intestinal microenvironment of broiler chickens. Poult. Sci. 100 (5), 103655. https://doi.org/10.1016/j.psj.2024.103655.
Islam, Z., Sultan, A., Khan, S., et al., 2024. Effects of an organic acids blend and coated essential oils on broiler growth performance, blood biochemical profile, gut health, and nutrient digestibility. Ital. J. Anim. Sci. 23 (1). https://doi.org/10.1080/1828051X.2023.2297562.

Jia, Z., Dumont, M.J., Orsat, V., 2016. Encapsulation of phenolic compounds present in plants using protein matrices. Food Biosci. 15 (1), 87–104. https://doi.org/10.1016/j.fbio.2016.05.007.

Kolososki, I.M.M., Saraiva, M.M.S., Nascimento, C.F., Campos, I.C., Lima, T.S., et al., 2024. Competitive exclusion products as an antimicrobial alternative to control *Salmonella* Heildelberg in broilers. L. Appl. Microb. 77 (8). https://doi.org/10.1093/lambio/ovae071.

Kumar, Y., Yadav, D.N., Ahmad, T., Narsaiah, K., 2015. Recent trends in the use of natural antioxidants for meat and meat products. Comp. Rev. F. Sci. F. Saf. 14 (1). https://doi.org/10.1111/1541-4337.12156.

Laborda, P., Garcia, F.S., Ochoa-Sanchez, L., et al., 2022. Wildlife and antibiotic resistance. Front. Vet. Sci. 12. https://doi.org/10.3389/fcimb.2022.873989.

Laptev, G.Y., Yildirim, E.A., Ilina, L.A., Filippova, V.A., Kochish, I.I., Gorfunkel, E.P., et al., 2021. Effects of essential oils-based supplement and *Salmonella* infection on gene expression, blood parameters, cecal microbiome, and egg production in laying hens. Animals. 11 (2), 360. https://doi.org/10.3390/ani11020360.

Li, Z.H., Cai, M., Liu, Y.S., Sun, P.L., Luo, S.L., 2019. Antibacterial activity and mechanisms of essential oil from *Citrus medica* L. var. *sarcodactylis*. Molecules 24 (8), 1577. https://doi.org/10.3390/molecules24081577.

Linh, N.T., Qui, N.H., Triatmojo, A., 2022. The effect of nano-ecapsulated herbal essential oils on poultry's health. Arch. Razi Inst. 77 (6), 2013–2021. https://doi.org/10.22092/ari.2022.358842.2318.

Liu, S., Song, M., Yun, W., Lee, J., Lee, C., Kwak, W., Han, N., Kim, H., Cho, J., 2018. Effects of oral administration of different dosages of carvacrol essential oils on intestinal barrier function in broilers. J. Anim. Physiol. Anim. Nutr. 102 (5), 1257–1265. https://doi.org/10.1111/jpn.12944.

Mariotti, M., Lombardini, G., Rizzo, S., et al., 2022. Potential applications of essential oils for environmental sanitation and antimicrobial treatment of intensive livestock infections. Microorganisms. 10 (4), 822. https://doi.org/10.3390/microorganisms10040822.

Maron, D.F., Smith, T.J.S., Nachman, K.E., 2023. Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. Glob. Health 9, 48. https://doi.org/10.1186/1744-8603-9-48.

Meenu, M., Padhan, B., Patel, M., et al., 2023. Antibacterial activity of essential oils from different parts of plants against *Salmonella* and *Listeria* spp. Food Chem. 404. https://doi.org/10.1016/j.foodchem.2022.134723.

Meira, N.V.B., Holley, R.A., Bordin, K., Macedo, R.E.F., Luciano, F.B., 2017. Combination of essential oil compounds and phenolic acids against *Escherichia coli* O157:H7 *in vitro* and in dry-fermented sausage production. Int. J. Food Microbiol. 2 (260), 59–64. https://doi.org/10.1016/j.ijfoodmicro.2017.08.010.

Meligy, A.M.A., El-Hamid, M.I.A., Yones, A.E., et al., 2023. Liposomal encapsulated oregano, cinnamon, and clove oils enhanced the performance, bacterial metabolites antioxidant potential, and intestinal microbiota of broiler chickens. Poult. Sci. 102 (6). https://doi.org/10.1016/j.psj.2023.102683.

Mo, K., Li, J., Liu, F., et al., 2022. Superiority of microencapsulated essential oils compared with common essential oils and antibiotics: effects on the intestinal health and gut microbiota of weaning piglet. Front. Nutr. 8 (12). https://doi.org/10.3389/fnut.2021.808106.

Moharreri, M., Vakili, R., Oskoueian, E., Rajabzadeh, G.H., 2022. Evaluation of microencapsulated essential oils in broilers challenged with *Salmonella* Enteritidis: a focus on the Body's antioxidant status, gut microbiology, and morphology. Arch. Raz. Inst. 77 (2), 629–639. https://dor.isc.ac/dor/20.1001.1.03653439.2022.77 .2.16.7.

Moreira, J.P.F.F., Monte, D.F.M., Lima, C.A., Oliveira, C.J.B., Martins, N.R.S., Berchieri Junior, A., Freitas Neto, O.C., 2022. Molecular genotyping reveals inter-regional relatedness among antimicrobial resistant *Salmonella* Minnesota strains isolated from poultry farm and humans, Brazil. Braz. J. Microbiol. 53 (1), 503–508. https://doi.org/10.1007/s42770-021-00666-1.

Movahedi, F., Nirmal, N., Wang, P., et al., 2024. Recent advances in essential oils and their nanoformulations for poultry feed. J. Anim. Sci. Biotechnol. 15, 110. https://doi.org/10.1186/s40104-024-01067-8.

Mustafa, A.A., Mirza, R.A., Aziz, H.I., 2023. Lavender essential oil in sanitization on fertile egg. Passer J. Basic Appl. Sci. 5 (2), 377–381. https://doi.org/10.24271/psr.2023.403768.1340.

Nameghi, A.H., Edalatian, O., Bakhshalinejad, R., 2019. Effects of a blend of thyme, peppermint and eucalyptus essential oils n growth performance, serum lipid and hepatic enzyme indices, immune response and ileal morphology and microflora in broilers. J. Anim. Physiol. Anim. Nutr. 103 (5), 1388–1398. https://doi.org/ 10.1111/jpn.13122.Nunez, ˜ K.V.M., Abreu, A.C.S., Almeida, J.M., et al., 2024. Antimicrobial activity of selected essential oils against *Staphylococcus aureus* from bovine mastitis. Dairy 5 (1), 54–65. https://doi.org/10.3390/dairy5010005.

Ocel’ova, V., Chizzola, R., Pisarcikova, J., Novak, J., Ivanisinova, O., Faix, S., 2016. Placha, I. Effect of thyme essential oil supplementation on thymol content in blood plasma, liver, kidney and muscle in broilers chickens. Nat. Prod. Com. 11 (10), 1545–1550.

Oliveira Filho, J.G.O., Duarte, L.G.R., Silva, Y.B.B., et al., 2023. Novel approach for improving papaya fruit storage with carnauba wax nanoemulsion in combination with *Syzigium aromaticum* and *Mentha spicata* Essential Oils. Coatings. https://doi.org/10.3390/coatings13050847.

Oliveira, G.S., McManus, C., Vale, I.R.R., et al., 2024. Obtaining microbiologically safe hatching eggs from hatcheries: using essential oils integrated sanitization strategies in hatching eggs, poultry houses and poultry. Pathogens 13 (3), 260. https://doi.org/10.3390/pathogens13030260.

Oliveira, G.S., Nascimento, S.T., Santos, V.M., et al., 2020. Clove essential oil in the sanitation of fertile eggs. Poult. Sci. 99 (11), 5509–5516. https://doi.org/10.1016/j.psj.2020.07.014.

Oliveira, G.S., McManus, C., Araújo, M.V., Sousa, D.E.R., Macedo, I.L., Castro, M.B., Santos, V.M., 2023. Sanitizing hatching eggs with essential oils: avian and microbiological safety. Microorg 11 (8), 1890. https://doi.org/10.3390/microorganisms11081890.

Penha Filho, R.A.C., Paiva, J.B., Silva, M.D., Almeida, A.M., Berchieri Jr, 2010. A. Control of *Salmonella* Enteritidis and *Salmonella* Gallinarum in birds by using live vaccine candidate containing attenuated *Salmonella* Gallinarum mutant strain. Vaccine. 28 (16), 2853–2859. https://doi.org/10.1016/j.vaccine.2010.01.058.

Penteado, A.L., Eschionato, R.A., Souza, D.R.C., et al., 2021. Avaliação *in vitro* de atividade antimicrobiana de óleos essenciais contra *Salmonella* Thyphimurium e *Staphylococcus aureus*. Rev. Hig. Aliment. 35 (293). https://doi.org/10.37585/HA2021.02avaliacao.

Pham, V.H., Abbas, W., Huang, J., et al., 2022. Effect of blending encapsulated essential oils and organic acids as an antibiotic growth promoter alternative on growth performance and intestinal health in broilers with necrotic enteritis. Poult. Sci. 101 (1), 101563. https://doi.org/10.1016/j.psj.2021.101563.

Ponomarenko, G.V., Kovalenko, V.L., Balatskiy, Y.O., et al., 2021. Bactericidal efficiency of preparation based on essential oils used in aerosol desinfection in the presence of poultry. Regul. Mech. Biosyst. 12 (4), 635–641. https://doi.org/10.15421/022187.

Porter, J.A., Morey, A., Manu, E.A., 2020. Antimicrobial efficacy of white mustard essential oil and carvacrol against *Salmonella* in refrigerated ground chicken. Poult. Sci. 99 (10), 5091–5095. https://doi.org/10.1016/j.psj.2020.06.027.

Puvaca, N., Lika, E., Tufarelli, V., et al., 2020. Influence of different tetracycline antimicrobial therapy of *Mycoplasma* (*Mycoplasma synoviae*) in laying hens compared to Tea Tree essential oil on table egg quality and antibiotics residues. Foods 9 (5), 612. https://doi.org/10.3390/foods9050612.

Rahman, M.R.T., Fliss, I., Biron, E., 2022. Insights in the development and uses of alternatives to antibiotic growth promoters in poultry and swine production. Antibiotics 11 (6), 766. https://doi.org/10.3390/antibiotics11060766.

Rapper, S.L., Viljoen, A., Vuuren, S.V., 2023. Optimizing the antimicrobial synergism of *Melaleuca alternifolia* (Tea Tree) essential oil combinations for application against respiratory related pathogens. Planta Med 89 (4), 454–463. https://doi.org/ 10.1055/a-1947-5680.

Robinson, K., Assumpcao, A.L.F.V., Arsi, K., Donoghue, A., Jesudhasan, P.R.R., 2022. Ability of garlic and ginger oil to reduce *Salmonella* in post-harvest poultry. Animals. 12 (21), 2974. https://doi.org/10.3390/ani12212974.

Roque-Borda, C.A., Saraiva, M.M.S., Monte, D.F.M., Rodrigues-Alves, L.B., Almeida, A. M., et al., 2022. HPMCAS-Coated alginate microparticles loaded with Ctx(Ile21)-Ha a promising antimicrobial agent against *Salmonella* Enteritidis in a chicken infection model. ACS Infec. Dis. 8 (1), 472–481. https://doi.org/10.1021/ acsinfecdis.1c00264.

Ruiz-Hernandez, K., Sosa-Morales, M.E., Ceron-García, ´ A., Gomez-Salazar, J.A., 2023. Physical, Chemical and sensory changes in meat and meat products induced by the addition of essential oils: a concise review. Food Rev. Int. 39 (4), 2027–2056. https://doi.org/10.1080/87559129.2021.1939369.

Saraiva, M.M.S., Lim, K., Monte, D.F.M., et al., 2022. Antimicrobial resistance in the globalized food chain: a One Health perspective applied to the poultry industry. Braz. J. Microbiol. 53 (1), 465–486. https://doi.org/10.1007/s42770-021-00635-8.

Saraiva, M.M.S., Moreira Filho, A.L.B., Freitas Neto, O.C., Silva, N.M.V., Givisiez, P.E.N., Gebreyes, W.A., Oliveira, C.J.B., 2018. Off-label use of ceftiofur in one-day chicks triggers a short-term increase of ESBL-producting *E. coli* in the gut. PLOS ONE. https://doi.org/10.1371/journal.pone.0203158.

Severino, R., Ferrari, G., Vu, K.D., et al., 2015. Antimicrobial effects of modified chitosan based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on green beans. Food Control 215–222. https://doi.org/10.1016/j.foodcont.2014.08.029.

Seyedtaghiya, M.H., Fasaei, B.N., Peighambari, S.M., 2021. Antimicrobial and antibiofilm effects of *Satureja hortensis* essential oil against *Escherichia coli* and *Salmonella* isolated from poultry. Iran. J. Microbiol 13 (1), 74–80. https://doi.org/ 10.18502/ijm.v13i1.5495.

Shayeganmehr, A., Marandi, M.V., Karimi, V., Barin, A., Ghalyanchilangeroudi, A., 2018. *Zataria multiflora* essential oil reduces replication rate of avian influenza virus (H9N2 subtype) in challenged broiler chicks. Immun. Heal. Dis. 59 (4), 389–395. https://doi.org/10.1080/00071668.2018.1478064.

Silva, F.V., Guimarães, A.G., Silva, E.R.S., et al., 2012. Anti-inflamatory and anti-ulcer activities of carvacrol, a monoterpene present in the essential oil of oregano. J. Med. Food 15 (11). https://doi.org/10.1089/jmf.2012.0102.

Sniegocki, T., Raszkowska-Kaczor, A., Bajer, K., Sell, B., Kozdrun, W., Giergiel, M., Posyniak, A., 2022. A preliminary study of the poultry body weight effect of carvacrol in litter and of carvacrol residue in organ tissue of exposed chickens. J. Vet. Res. 66 (4), 613–617. https://doi.org/10.2478/jvetres-2022-0054.

Somrani, M., Debbabi, H., Palop, A., 2022. Antibacterial and antibiofilm activity of essential oil of clove against *Listeria monocytogenes* and *Salmonella* Enteritidis. Food Sci. Technol. Int. 28 (4), 331–339. https://doi.org/10.1177/10820132211013273.

Sousa, D.P., Damasceno, R.O.S., Amorati, R., Elshabrawy, H.A., Castro, R.D., et al., 2023. Essential oils: chemistry and pharmacological activities. Biom 13 (7), 1144. https://doi.org/10.3390/biom13071144.

Souza, A.I.S., Saraiva, M.M.S., Casas, M.R.T., Oliveira, G.M., Cardozo, M.V., Benevides, V.P., Barbosa, F.O., Freitas Neto, O.C., Almeida, A.M., Berchieri Junior, A., 2020. High occurrence of β-lactamase-producing *Salmonella* Heidelberg from poultry origin. PLOS ONE. https://doi.org/10.1371/journal.pone.0230676.

Stingelin, G.M., Scherer, R.S., Machado, A.C., et al., 2023. The use of thymol, carvacrol and sorbic acid in microencapsules to control *Salmonella* Heidelberg, *S*. Minnesota and *S*. Thypimurium in broilers. Front. Vet. Sci. https://doi.org/10.3389/ fvets.2022.1046395.

Sundar, S.K., Parikh, J.K., 2023. Advances and trends in encapsulation of essential oils. Int. J. Pharm. 635 (25). https://doi.org/10.1016/j.ijpharm.2023.122668.

Szczepanik, M., Raszkowska-Kaczor, A., Olkiewicz, D., Bajer, D., Bajer, K., 2020. Use of starch granules enriched with carvacrol for the lesser mealworm, *Alphitobius diaperinus* control in chicken house: effects on insects and poultry. J. Poult. Sci. 57 (2), 168–174. https://doi.org/10.2141/jpsa.0190068.

Tebrün, W., Motola, G., Hafez, M.H., Bachmeier, J., Schmidt, V., Renfert, K., Reichelt, C., Bruggemann-Schwarze, S., Pees, M., 2020. Preliminary study: health and performance assessment in broiler chicks following application of six different hatching egg disinfection protocols. Plos One. https://doi.org/10.1371/journal. pone.0232825.

Thomas, C.M., Nielsen, K.M., 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat. Rev. Microbiol. 3 (1), 711–721. https://doi.org/ 10.1038/nrmicro1234.

Trivino, F.J.N., Cuenca-Barrales, C., Ruiz-Villaverde, R., 2019. Eugenol allergy mimicking recurrent aphthous stomatitis and burning mouth syndrome. Contact Dermat. 81 (6), 462–463. https://doi.org/10.1111/cod.13365.

Turgis, M., Han, J., Caillet, S., Lacroix, M., 2009. Antimicrobial activity of mustard essential oil against *Escherichia coli* O157:H7 and *Salmonella* typhi. Food Control 20 (12), 1073–1079. https://doi.org/10.1016/j.foodcont.2009.02.001.

Van Boeckel, T.P., Brower, C., Gilberto, C., et al., 2015. Global trends in antimicrobial use in food animals. PNAS 112 (18), 5649–5654. https://doi.org/10.1073/ pnas.1503141112.

Visvalingam, J., Palaniappan, K., Holley, R.A., 2017. *In vitro* enhancement of antibiotic susceptibility of drug-resistant *Escherichia coli* by cinnamaldehyde. Food Control 79 (1), 288–291. https://doi.org/10.1016/j.foodcont.2017.04.011.

Wang, H., Liang, S., Li, X., Yang, X., et al., 2019. Effects of encapsulated essential oils and organic acids on laying performance, egg quality, intestinal morphology, barrier function, and microflora count of hens during the early laying period. Poult. Sci. 98 (12), 6751–6760. https://doi.org/10.3382/ps/pez391.

Xu, X., Zhou, W., Xie, C., et al., 2022. Airborne bacterial communities in the poultry farm and their relevance with environmental factors and antibiotics resistance genes. Sci. Total Environ. 846, 157420. https://doi.org/10.1016/j.scitotenv.2022.157420.

Yilmaz, E.A., Yalçin, H., Polat, Z., 2024. Antimicrobial effects of laurel extract, laurel essential oil, zahter extract, and zahter essential oil on chicken wings contaminated with *Salmonella* Typhimurium. Vet. Med. Sci. 10, e1445. https://doi.org/10.1002/ vms3.1445.

Yuan, C., Wang, Y., Liu, Y., Cui, B., 2019. Physicochemical characterization and antibacterial activity assessment of lavender essential oil encapsulated in hydroxypropyl-beta-cyclodextrin. Ind. Crops Prod. 130 (1), 104–110. https://doi. org/10.1016/j.indcrop.2018.12.067.

Zhai, H., Liu, H., Wang, S., et al., 2018. Potential of essential oils for poultry and pigs. Anim. Nutr. 4 (2), 179–186. https://doi.org/10.1016/j.aninu.2018.01.005.

Zhang, L.Y., Peng, Q.Y., Liu, Y.R., et al., 2021a. Effects of oregano essential oil as an antibiotic growth promoter alternative on growth performance, antioxidant status, and intestinal health of broilers. Poult. Sci. 100. https://doi.org/10.1016/j. psj.2021.101163.

Zhang, S., Shen, Y.R., Wu, S., Xiao, Y.Q., et al., 2019. The dietary combination of essential oils and organic acids reduces *Salmonella* Enteritidis in challenged chicks. Poult. Sci. 98 (12), 6349–6355. https://doi.org/10.3382/ps/pez457.

Zhang, X., Peng, Y., Wu, C., 2021b. Chicken embryonic toxicity and potential *in vitro* estrogenic and mutagenic activity of carvacrol and thymol in low dose/ concentration. Food Chem. Toxicol. 150 (1), 112038. https://doi.org/10.1016/j.fct.2021.112038.
Zhang, Y., Li, X.Y., Zhang, B.S., et al., 2022. *In vivo* antiviral effect of plant essential oils against avian infectious bronchitis virus. BMC Vet. Res. 18, 90. https://doi.org/10.1186/s12917-022-03183-x.

CAPÍTULO 3 – Effective Control of *Salmonella* Enteritidis in Poultry by Dietary Supplementation with Microencapsulated Essential Oils[2]

Heitor Leocádio de Souza Rodrigues[1†], Valdinete Pereira Benevides[1†], Isis Mari Miyashiro Kolososki[1], Mauro M. S. Saraiva[1], Nayla Pádua Del Bianco Gontijo Souki[2], Tarley Araújo Barros[2], André Luis Costa Rabelo[1], Viviane Amorim Ferreira[1], Melissa Freitas Feitosa Dix[1], Adriana Maria Almeida[1], Cesar Augusto Roque-Borda[3*], Angelo Berchieri Junior[1*].

[1]School of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal 14884-900, Brazil; [2]Agroceres Multimix Animal Nutrition, Rio Claro 13502-741, Brazil; [3]Vicerrectorado de Investigación, Universidad Católica de Santa Maria, Arequipa 04000, Peru; †These authors contributed equally to this work; *Authors to whom correspondence should be addressed (roque.cesar@gmail.com and angelo.berchieri@unesp.br).

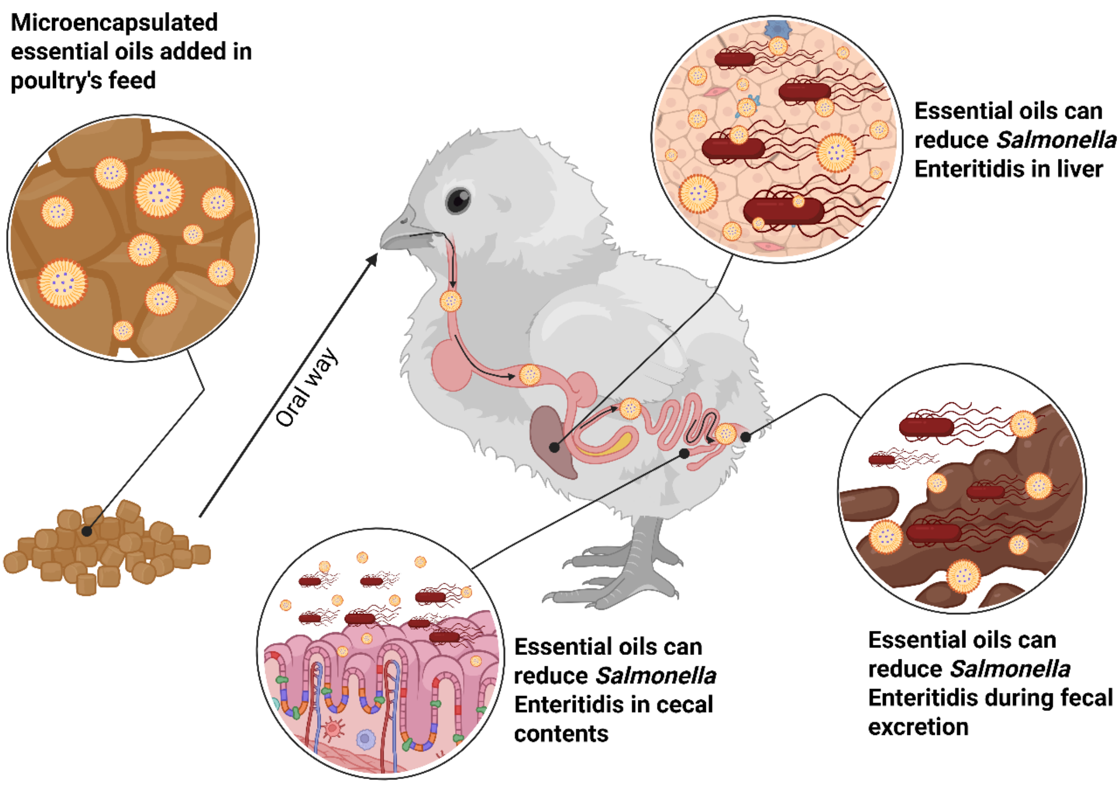
Abstract

Background/Objectives: *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) is a major pathogen associated with poultry products, and the rise of antimicrobial-resistant strains has intensified the need for effective natural control strategies. Essential oils (EOs) are recognized for their antimicrobial potential, but their volatility, instability, and risk of toxicity at high concentrations limit their practical application. Therefore, the objective of this study was to evaluate the antimicrobial efficacy of EOs in broilers infected with *S.* Enteritidis and to characterize potential synergistic or antagonistic interactions between the oils. **Methods:** To achieve this, the oils were first assessed through Minimum Inhibitory Concentration (MIC), Minimum

[2] Este capítulo corresponde ao artigo científico publicado na revista *Antibiotics* em 2025.

Bactericidal Concentration (MBC), and Fractional Inhibitory Concentration (FIC) assays, and the most effective ones against *S*. Enteritidis were selected. These selected oils were then microencapsulated and incorporated into the broiler feed for the in vivo assay. **Results:** The encapsulated formulation retained key bioactive compounds and significantly reduced bacterial shedding and intestinal colonization when administered to broilers experimentally infected with *S*. Enteritidis. Broilers receiving the optimized half-dose supplementation exhibited a 36% reduction in fecal shedding and a 4 $\log_{10}$ decrease in cecal bacterial counts compared to untreated controls. A transient reduction in liver colonization was also observed, while feed intake remained unaffected. **Conclusions:** These findings demonstrate that microencapsulated EOs can serve as an effective natural strategy to control *S.* Enteritidis in poultry. The results support the broader application of lipid-based encapsulation technologies for improving the functional performance of phytobiotics in animal production.
Keywords: cinnamon; clove; oregano; phytobiotics; poultry farm; synergistic effect.

Graphical Abstract



1. Introduction

Salmonella enterica subsp. *enterica* serovar Enteritidis (*S*. Enteritidis) is among the most frequently isolated paratyphoid serovars in poultry products and human foodborne infections [1]. Although infection in poultry is often asymptomatic, the bacterium is still excreted and disseminated within and beyond the poultry environment [2]. In addition to its rapid spread, antimicrobial-resistant or multidrug-resistant *Salmonella enterica* strains are frequently isolated from poultry production, likely due to the indiscriminate use of antimicrobials, posing a challenge to One Health [3,4,5].

Given the need to control the spread of *Salmonella* strains and the restrictions on conventional antimicrobials in poultry farming, novel antimicrobial products have been investigated worldwide. Among the natural alternatives, essential oils (EOs) have been proposed due to their reported antimicrobial activity against various avian paratyphoid *Salmonella* strains [6,7]. Different applications of these phytobiotics have been suggested in poultry farming, including carcass decontamination [8], performance enhancement in broilers [9], litter sanitization [10], egg disinfection [11], and antimicrobial agents to control *Salmonella* in poultry [12]. Although their precise mechanisms of action have not been fully elucidated, the antimicrobial activity of EOs is hypothesized to be associated with their interaction with the bacterial cell wall [13,14]. This effect may be even more potent when EOs are synergistically combined with conventional antimicrobials or other EOs, enhancing the efficacy of one or both agents, such as tetracycline and thyme [15].

Some limitations associated with the use of EOs as antimicrobials in poultry include high volatility, sensitivity to oxygen and/or luminosity, low water solubility, and rapid degradation in the upper gastrointestinal tract [16]. To mitigate these challenges, EO encapsulation technologies have been proposed as a promising approach to reduce volatilization, enhance bioavailability, and improve the delivery of these compounds within the host organism [17]. Microencapsulation allows the active compounds to be embedded within a protective matrix, reducing evaporation and degradation while facilitating controlled release at target sites, such as the intestinal tract [18].

Lipid-based matrices have shown favorable physicochemical compatibility with EO components, making them suitable carriers for feed applications [19,20,21,22].

Additionally, encapsulation can enhance storage stability, mask strong odors, and minimize adverse effects related to EO overdosing [23,24,25]. In this context, the present study aimed to evaluate the antimicrobial efficacy of microencapsulated EOs against *S.* Enteritidis in experimentally infected poultry and characterize the occurrence of synergism or antagonism among phytobiotics in vitro.

2. Results

2.1. In Vitro Results

Among the EOs evaluated, cinnamon EO exhibited the strongest antimicrobial activity against *S.* Enteritidis, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 0.22 and 0.44 mg/mL, respectively. Oregano (MIC: 0.49 mg/mL), white thyme (0.57 mg/mL), clove (0.69 mg/mL), and mentha (0.69 mg/mL) also demonstrated significant inhibitory effects. In contrast, no inhibitory activity was detected for black pepper, ginger, copaiba, or sweet pear orange EOs, even at a maximum tested concentration of 10% (*v*/*v*) (Table 1).

Table 1. MIC and MBC values of tested EOs against *S.* Enteritidis.

EOs	MIC (mg/mL)	MBC (mg/mL)
Oregano	0.49	0.99
Cinnamon	0.22	0.44
Clove	0.69	2.76
Bay laurel	4.56	4.56
Citronella	4.48	4.48
White thyme	0.57	2.28
Lemongrass	2.25	2.25
Rosemary	11.52	23.05
Tea tree	1.37	1.37
Black pepper*	-	-
Eucalyptus	5.83	23.32
Ginger*	-	-
Basil	5.79	23.17
Copaiba*	-	-

Sweet pear orange*	-	-
Mentha	0.69	2.77

*: no effect against S. Enteritidis was observed at a 10 % (*v/v*) concentration of EOs.

Bactericidal activity, as indicated by MBC values, varied among the EOs. While cinnamon, oregano, and tea tree oils eliminated the pathogen at relatively low concentrations, other EOs — including basil, mentha, clove, white thyme, and eucalyptus — required concentrations four times higher than their respective MICs to achieve the same effect. These findings underscore a diverse range of bactericidal potential among the tested oils. All MIC and MBC values are summarized in Table 1.

In addition to individual efficacy, selected EOs were evaluated in combination to determine potential interactive effects against *S.* Enteritidis. Among the top-performing EOs — cinnamon, clove, oregano, white thyme, and mentha — synergistic, commutative, indifferent, and antagonistic interactions were observed, as detailed in Table 2. A synergistic interaction was identified between mentha and oregano, where the MIC values of mentha and oregano were reduced by four- and two-fold, respectively, yielding a Fractional Inhibitory Concentration Index (FICI) of 0.75. In contrast, combinations of mentha with white thyme or cinnamon resulted in antagonistic effects, with FICI values of 8 and 6, respectively, and a four-fold increase in their MICs related to the individual oils. Several pairings — including oregano/cinnamon, oregano/white thyme, cinnamon/clove, white thyme/clove, and mentha/clove — exhibited commutative effects (FICI = 2), indicating additive but non-synergistic interactions. The combination of oregano and clove was classified as indifferent (FICI = 1), suggesting that the combined effect was neither enhanced nor diminished relative to the individual activities.

Table 2. Fractional Inhibitory Concentration Index (FICI) and interaction classification of selected essential oil combinations against *Salmonella* Enteritidis.

EOs combinations	**FICI**	**Classification**
Oregano / Cinnamon	2	Commutative
Oregano / White thyme	2	Commutative
Cinnamon / White thyme	4	Antagonistic
Cinnamon / Clove	2	Commutative

Oregano / Clove	1	Indifferent
White thyme / Clove	2	Commutative
Mentha / White thyme	8	Antagonistic
Mentha / Clove	2	Commutative
Mentha / Cinnamon	6	Antagonistic
Mentha / Oregano	0.75	**Synergistic**

2.2. Encapsulation and Quantification of Active Compounds

Following the identification of synergistic effects between mentha and oregano EOs, a lipid-based encapsulation system was developed to enhance the stability of the bioactive compounds. For this, a hydrophobic matrix was employed to encapsulate a blend composed of cassia cinnamon, oregano, and clove EOs. The selected mass proportions were 92.26 g (cassia cinnamon), 104.84 g (oregano), and 146.77 g (clove), totaling 343.9 g of EOs. The encapsulation matrix consisted predominantly of fat (86%), with the EO blend comprising 11% of the total mass and silica acting as an adsorbent at 3%. The final powdered product reached a total mass of 3194.88 g. This approach aimed to protect the active compounds from volatilization and degradation.

The selection of cassia cinnamon, oregano, and clove EOs for encapsulation was based on their individual antimicrobial activity against *S.* Enteritidis, the chemical stability of their major constituents, and their compatibility with lipid-based matrices. Although the combination of mentha and oregano demonstrated in vitro synergism, mentha EO was excluded from the encapsulated formulation due to its antagonistic interaction with cassia cinnamon and white thyme EOs.

The average particle diameter of the microcapsules was 28.34 ± 2.65 μm (n = 20), based on the mean of two perpendicular measurements per capsule. The observed values ranged from 25.45 to 33.81 μm. These results indicate a relatively uniform size distribution of the lipid-encapsulated EO particles (Figure S1).

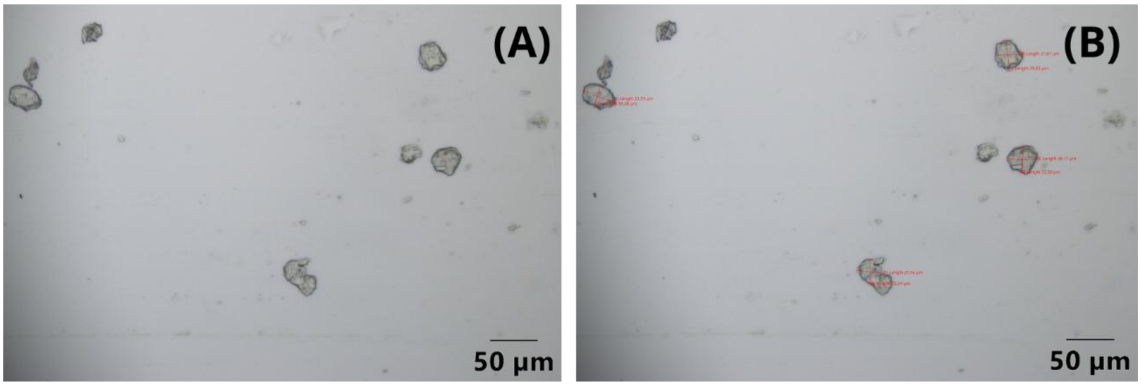


Figure S1. Optical microscopy of the microcapsules present in the EO blend under a 20× objective lens. (A) Microcapsules; (B) Measurement of orthogonal diameters (length and width) using CellSens Standard© software, version 4.2 (CS-ST-V4.2).

High-Performance Liquid Chromatography (HPLC) analysis was performed to assess the retention of active constituents' post-encapsulation (Figure 1). The quantified markers included carvacrol and thymol (from oregano oil), eugenol (from clove oil), and cinnamaldehyde (from cinnamon oil). In the unencapsulated blend, the concentrations of carvacrol, thymol, eugenol, and cinnamaldehyde were 20.7%, 3.98%, 35.3%, and 19.9%, respectively (Table 3). Following encapsulation, the corresponding values decreased to 2.02%, 0.39%, 3.49%, and 1.99%, reflecting an approximately 10-fold dilution. It is important to clarify that the observed decrease in the concentrations of carvacrol, thymol, eugenol, and cinnamaldehyde post-encapsulation (approximately 10% of their initial values) reflects the dilution effect resulting from the incorporation of the essential oil blend into a much larger lipid matrix. This reduction does not represent compound degradation or poor recovery during encapsulation. Due to confidentiality agreements with the encapsulating company, quantitative recovery data (e.g., encapsulation efficiency) could not be disclosed. However, the consistent biological activity observed in vivo indicates that a functionally relevant proportion of the active compounds was retained and effectively delivered. This is consistent with previous reports describing encapsulation efficiencies ranging from 20% to 85% for lipid-based systems, depending on formulation strategies and analytical conditions.

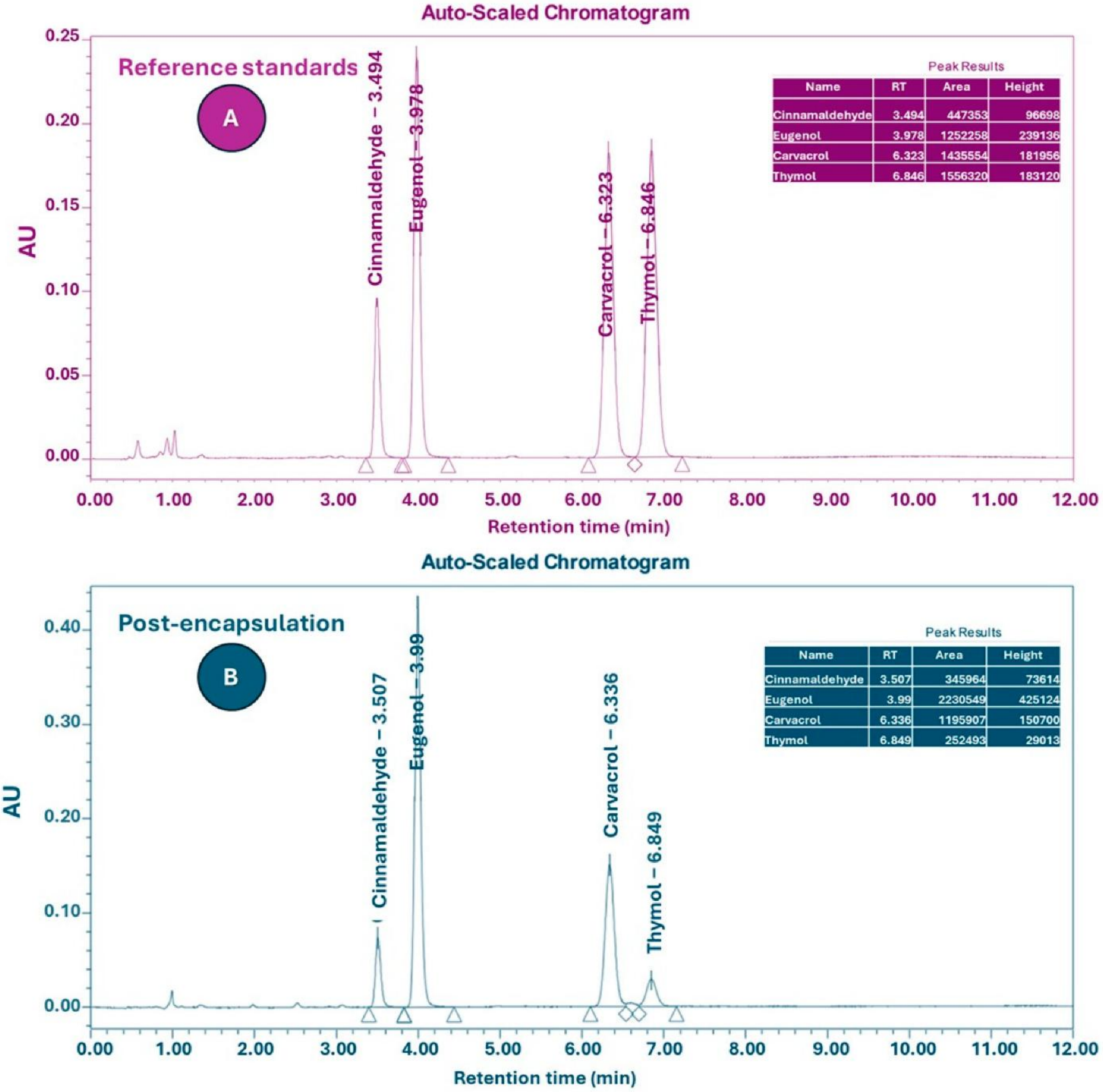


Figure 1. (**A**) HPLC retention profiles of analytical markers: cinnamaldehyde, eugenol, carvacrol, and thymol. (**B**) Chromatographic profile of the encapsulated EO blend analyzed using HPLC. Detection performed at 203 nm; major peaks correspond to retained active compounds within the lipid matrix.

Due to the different dilution factors applied to the sample and standards, the peak areas were not directly proportional. Therefore, the final percentages shown in Table **3** reflect concentration values corrected by both the dilution factors and standard curves, rather than raw or normalized peak area ratios.

Table 3. Percentage of active compounds in the EO blend (cinnamon + clove + oregano EO) before and after encapsulation.

Compounds	Before encapsulation (%)	Post-encapsulation (%)
Carvacrol	20.7	2.02
Thymol	3.98	0.39
Eugenol	35.3	3.49
Cinnamaldehyde	19.9	1.99

Based on the concentration of active compounds in the encapsulated product, the recommended inclusion level in animal feed was calculated as 41 g/kg. This corresponds to 1.1 g of cinnamon oil, 1.25 g of oregano oil, and 1.75 g of clove oil per kg of feed, ensuring delivery of 4.1 g of the EO blend per kg. The encapsulated product was protected from light, heat, and moisture to maintain stability until use.

2.3. Chick In Vivo Results

To investigate the in vivo antimicrobial efficacy of the microencapsulated EO blend, a broiler infection model was used, involving oral challenge with *S*. Enteritidis. The EO blend — comprising cassia cinnamon (1.1 g/kg), clove (1.75 g/kg), and oregano (1.25 g/kg) — was administered in feed at two concentrations. HPLC analysis previously confirmed that encapsulation retained approximately 10% of the initial concentration of active compounds (see Section 2.2.).

Fecal Shedding —Cloacal swab analysis revealed significant differences in *S.* Enteritidis fecal excretion across groups (Figure 2). Group A exhibited a 54.44% positivity rate (49/90), substantially lower than the 77.77% in group B (70/90) and 90% in group C (81/90). The 36% fewer positive swabs from fecal excretion in group A relative to the control was statistically significant ($p < 0.05$), indicating that the lower EO dose was more effective at limiting pathogen shedding.

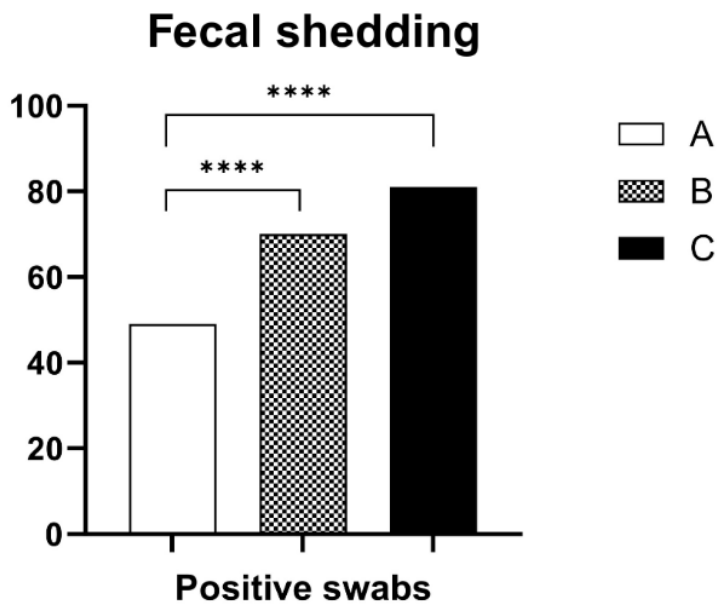


Figure 2. Fecal shedding of *S.* Enteritidis during 21 days of the experiment. Groups A (half dose of EO blend), B (full dose of EO blend), and C (non-treatment). A = 54.44% positive swabs for *S.* Enteritidis; B = 77.77%; C = 90%. **** High statistical difference between groups A/B and A/C.

Cecal Colonization — Bacterial enumeration in cecal contents (Figure 3I) confirmed the superior effect of the lower EO dose. At 7 dpi, group A chicks presented a significant 4 $\log_{10}$ reduction in *S.* Enteritidis counts compared to group C ($p < 0.01$). This effect remained statistically significant at 14 dpi ($p < 0.01$) and persisted through 21 dpi ($p < 0.05$), demonstrating a sustained inhibitory effect of the microencapsulated EOs on intestinal colonization. In contrast, group B showed no significant improvement compared to the control.

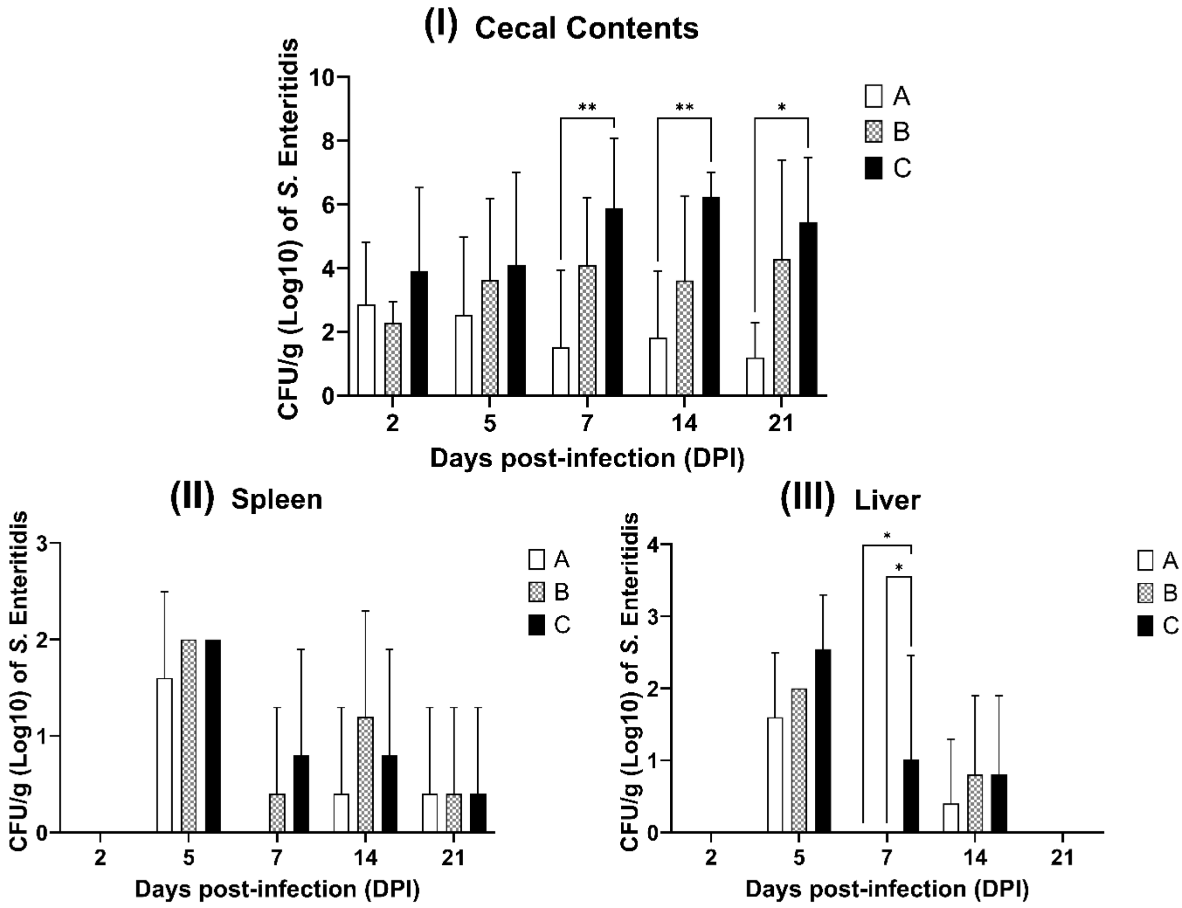


Figure 3. The viable CFU/g of cecal contents from chickens inoculated with *S.* Enteritidis (SE P125 109): (**I**); spleen (**II**); and liver (**III**). Group A (half dose of EO blend), group B (full dose of EO blend), and group C (non-treatment). * ($p < 0.05$); ** ($p < 0.01$); Statistical difference in comparison between groups A, B, and C using two-way ANOVA with Bonferroni's Multiple Comparisons test at 5% probability.

Systemic Dissemination: Liver and Spleen — Bacterial translocation to systemic organs was evaluated by enumerating CFUs in liver and spleen samples (Figure 3II,III). In the spleen, no significant differences in bacterial load were observed among groups at any time point. In the liver, however, a notable transient effect was detected at 7 dpi. Both groups A and B demonstrated significantly lower *S.* Enteritidis counts compared to group C ($p < 0.05$). While the reduction was more pronounced in group A, no significant differences were observed among groups at 14 and 21 dpi.

Daily feed Intake —Daily feed intake measurements (Figure 4) revealed no statistically significant differences between treated and untreated groups throughout the 23-day experiment. All groups followed a similar feed intake trajectory, indicating that the inclusion of the EO blend, regardless of concentration, did not impair feed palatability or broiler appetite. This supports the potential feasibility of EO inclusion in poultry diets from a production standpoint.

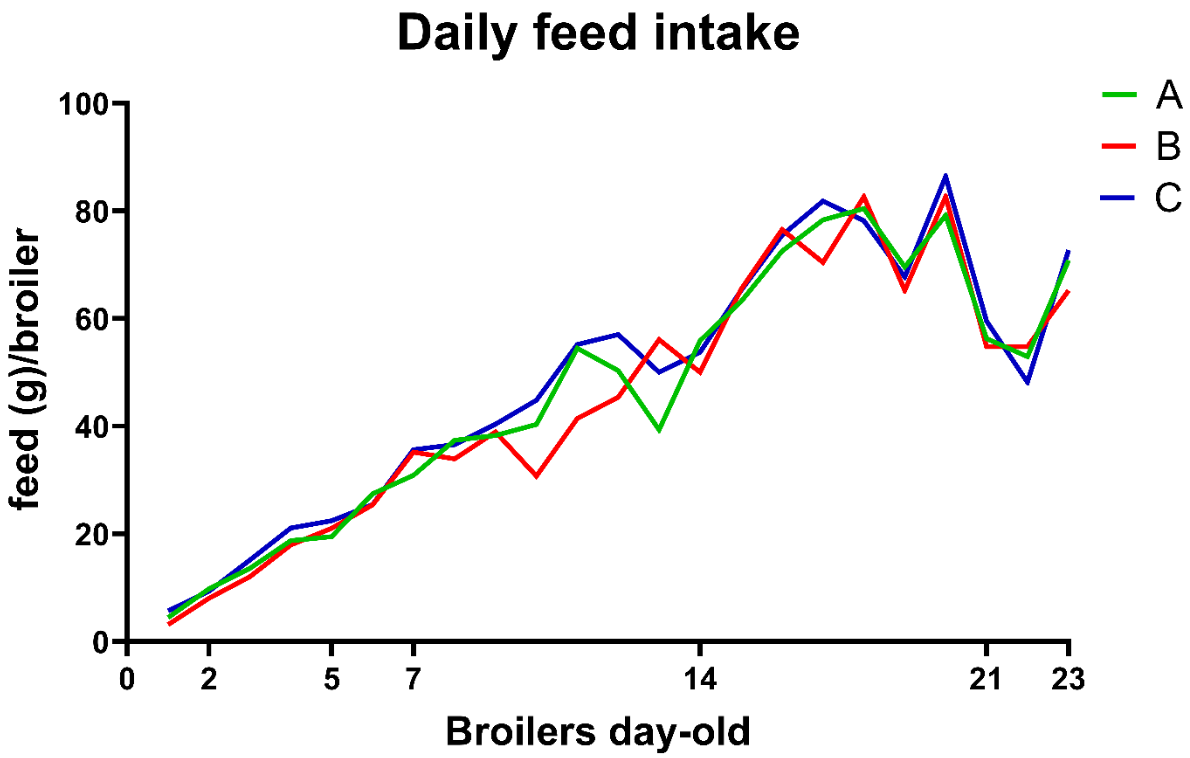


Figure 4. Average daily feed intake of broilers of groups A (half dose of EO blend), B (full dose of EO blend) and C (non-treatment).

Although no histological evaluation was performed, necropsy findings provided valuable insights into tissue-level responses. Macroscopic examination of all broilers from group B (7–14 dpi) revealed signs of intestinal mucosal congestion, hepatic discoloration, and yolk sac retention in several animals. These lesions were not observed in group A and were minimal in group C.

3. Discussion

EOs derived from culinary herbs — such as cinnamon, clove and oregano — have long been utilized for their antimicrobial properties, particularly in food preservation and safety applications [26]. Their increasing use as phytobiotics in poultry production aims to control enteric pathogens, such as *Salmonella enterica*, while minimizing antibiotic reliance [16]. However, their practical use is often limited by volatility, poor solubility, and challenges in maintaining effective concentrations at target sites. Microencapsulation technologies have emerged as a strategic solution, allowing gradual release along the gastrointestinal tract and improved systemic bioavailability [27]. This is especially relevant considering that non-encapsulated EO treatments often show lower reductions in cecal and cloacal colonization by *Salmonella* [12,28].

Our results confirm the in vitro activity of certain EOs against *S.* Enteritidis, with cinnamon EO demonstrating the strongest inhibitory and bactericidal activity (MIC = 0.22 mg/mL). This result aligns with previous findings attributing cinnamon's efficacy to cinnamaldehyde, a major active compound known to compromise bacterial membrane integrity and modulate gene expression [7,16,29]. Oregano, white thyme, mentha, and clove also displayed relevant antimicrobial effects, in line with reports documenting their phenolic components — such as thymol, carvacrol, menthol, and eugenol — as responsible for disrupting bacterial homeostasis [30]. In contrast, black pepper, ginger, copaiba, and sweet pear orange EOs exhibited no inhibitory activity against *S.* Enteritidis under the tested conditions, despite previous reports of their antimicrobial efficacy against other pathogens [8,31]. These discrepancies highlight the strain-specific nature of EO activity, which can be influenced by factors such as bacterial membrane composition, pH, emulsification capacity, and oil solubility [32].

The bactericidal activity profile revealed that all inhibitory EOs could eliminate *S.* Enteritidis at concentrations ranging from 1× to 4× MIC, consistent with earlier studies showing similar MIC – MBC relationships for *Salmonella* spp. [6,33,34]. This suggests that although some EOs act as bacteriostatic agents at low concentrations, increasing the dose can shift their activity toward bactericidal effects, an important consideration for dosage optimization in feed applications. Moreover, we highlight that the study's strength lies in the combinatorial evaluation of EOs interactions through FICI analysis — an approach seldom addressed in poultry or veterinary microbiology trials despite the common use of EO blends — and, notably, a synergistic interaction was observed between mentha and oregano EOs, resulting in substantial MIC reduction.

On the other hand, antagonistic effects were observed in combinations such as mentha with white thyme or cinnamon, where the MICs of both components increased significantly. This highlights a critical but often overlooked issue: the empirical mixing of

EOs without mechanistic compatibility testing may lead to reduced effectiveness, or even counterproductive interactions. This reinforces prior warnings against arbitrary EO combinations [16] and emphasizes the need for rational formulation design; also, the indifferent interaction observed between oregano and clove (FICI = 1) and the predominance of commutative (additive) effects further underscore the complexity of EO interactions. Interestingly, while clove and cinnamon exhibited additive behavior in this study, previous research has shown them to be synergistic or antagonistic depending on the target microorganism [35,36]. For instance, synergy was reported against *S.* Typhimurium and *Aspergillus niger*, whereas antagonism was noted against *Escherichia coli* but not *Listeria monocytogenes* or *Yersinia enterocolitica*. These observations support the notion that EO interactions are highly context-dependent, influenced by microbial physiology, EO composition, and testing conditions [37], which can pose difficulty in the use of EOs. Overall, the initial findings of this study provide mechanistic and practical insights into the antimicrobial potential of individual and combined EOs. While some combinations can significantly enhance activity through synergism, others may compromise efficacy due to antagonistic interactions.

Although mentha EO demonstrated in vitro synergism with oregano, it was excluded from the final EO blend due to antagonistic interaction with cinnamon. This decision was based on preliminary FICI results that indicated a reduced antimicrobial effect when mentha and cinnamon were combined. Nevertheless, this exclusion may have overlooked potentially effective combinations, such as mentha + oregano without cinnamon. Future studies should consider testing alternative binary or ternary EO blends to further optimize antimicrobial efficacy while avoiding antagonistic interactions.

The encapsulation approach applied in this study represents a critical technological intervention to enhance the usability of bioactive compounds in animal nutrition and food science [38]. By embedding the bioactive compound blend into a lipid or polymeric matrix, the strategy aimed not only to reduce volatility and degradation but also to enable site-specific release in the intestinal tract — a key requirement for targeting enteric pathogens such as *Salmonella* sp. [18,39,40]. Rather than focusing on the quantitative loss during encapsulation — an inherent characteristic of the dilution process — this study demonstrates that biological efficacy can still be preserved even when active compound concentrations are significantly reduced. Although we did not assess the controlled release of the EO blend in this study, previous research using similar capsules has reported that their degradation occurs gradually along the gastrointestinal tract [23,24,25]. The observed antimicrobial performance in vivo, particularly in group A, supports the hypothesis that controlled release and local delivery may compensate for lower nominal concentrations by improving compound stability and bioaccessibility at the site of infection [41].

The formulation, based on hydrogenated vegetable fat and silica, enables cold dispersion in feed mixtures and offers protection during storage and thermal processing, and these attributes are particularly relevant for industrial-scale feed production, where compound volatility and oxidative instability typically limit the phytogenic use [42]. Although the process preserved four key analytical markers (carvacrol, thymol, cinnamaldehyde, and eugenol), it is plausible that minor volatile or synergistic compounds were lost or chemically altered during encapsulation, potentially modifying the overall spectrum of activity [43]. In the present study, the microcapsules produced had an average diameter of 28 µm, which is considerably smaller than those reported in previous research [38,44], where capsule sizes reached approximately 32–2000 µm for poultry administration. Smaller particle sizes, such as those obtained in our formulation, may offer advantages in terms of more uniform blending into feed and easier ingestion by broilers, potentially improving the distribution and bioavailability of the active compounds [45,46]. Although exact encapsulation efficiency values could not be disclosed due to confidentiality agreements with the partnering company, the observed antimicrobial efficacy in vivo supports the hypothesis that the major bioactive compounds were adequately preserved and delivered. The apparent reduction in compound concentration to approximately 10% of the original values was due to intentional dilution in the lipid matrix, not compound loss. This aligns with previous reports showing encapsulation recoveries ranging from 20% to 85% in lipid- and polymer-based systems [23,24,25,38,44], indicating that the formulation applied here falls within the expected range for effective encapsulation in animal feed applications.

For the in vivo trial, a full dose of EOs was selected compared to those typically reported in poultry studies [9,12,47,48] to evaluate anti-*Salmonella* efficacy at elevated doses. While most studies focusing on growth performance use EO blends at 150–800 mg/kg [9,12], antimicrobial effects have been observed with much lower concentrations, such as 50–100 mg/kg of cinnamaldehyde or thymol, which reduced *S.* Enteritidis prevalence by up to 25% [49]. Another study combining 45 mg/kg of carvacrol, 100 mg/kg of cinnamaldehyde, 200 mg/kg of cineole, and 2 g/kg of capsaicin achieved a 3-log reduction in *S.* Enteritidis [28]. These findings suggest that concentrations ranging from 100 to 600 mg/kg per EO may be sufficient for pathogen control in poultry, although outcomes may vary depending on environmental challenges and the bioavailability of the active compounds in the host organism [50].

In our study, supplementation with the EO blend significantly reduced fecal shedding of *S.* Enteritidis by approximately 36% compared to the untreated control. This result is consistent with previous reports showing reductions in the shedding of *S.* Heidelberg, *S.* Minnesota, and *S.* Typhimurium following treatment with EOs and organic acids [51]. These findings underscore the potential of EO-based strategies to decrease horizontal transmission of paratyphoid *Salmonella* serovars in poultry production systems. A notable finding was the significant 4 $\log_{10}$ reduction in cecal *S.* Enteritidis counts observed in broilers treated with half the recommended EO dose. This reduction was evident from 7 dpi and persisted through 21 dpi. Similar time-dependent antimicrobial effects have been reported in other studies, including reductions observed at 7 and 14 dpi [51] and even earlier, as reported by Hu et al. [12], where declines began at 3 dpi and extended to 10 dpi. These results reinforce the importance of defining an optimal dose–response curve to guide the future standardization of EO-based antimicrobials for poultry applications. Although the EO blend significantly reduced cecal bacterial load, no significant changes were observed in spleen colonization. However, a transient decrease in liver colonization was noted at 7 dpi in the treated groups. This may reflect a reduction in the translocation of bacteria from the intestine to systemic organs, possibly due to improvements in gut integrity and microbial balance promoted by EOs [12].

Interestingly, the most pronounced antimicrobial effect was observed at the lower dose of the EO blend. This paradox may be explained by the non-selective antimicrobial properties of EOs, which, at higher concentrations, could disrupt the intestinal microbiota and damage the mucosal barrier. Supporting this hypothesis, Laptev et al. [52] reported marked shifts in the gut microbiota of EO-treated chickens infected with *S.* Enteritidis, including reductions in beneficial genera such as *Bifidobacterium* and *Lactobacillus*.

These findings suggest that high EO doses may inadvertently compromise gut health and indicate potential dose-dependent toxicity associated with higher EO concentrations, aligning with previous reports of mucosal irritation or microbiota disruption linked to phenolic EO components [53,54,55]. The lower effectiveness and apparent adverse effects in group B may reflect the non-selective antimicrobial nature of the blend at excessive doses, potentially leading to gut dysbiosis or epithelial damage [56]. Daily feed intake remained unaffected by EO supplementation, indicating good palatability and compatibility of the blend at both tested concentrations. Similar results have been reported in the literature [9,57]. Although this study did not include zootechnical performance assessments due to the infection-focused design, prior research has shown that EO supplementation can improve the feed conversion ratio, enhance gut morphology, and reduce carcass fat content in broilers [9,58].

A key limitation of this study is the extrapolation of in vitro findings to in vivo conditions, which involve complex physiological variables such as digestive enzymatic activity, pH fluctuations, and interactions with the gut microbiota — factors that can significantly influence the efficacy of EOs. Moreover, the observed signs of mucosal and hepatic damage in broilers administered the higher EO dose highlight the importance of comprehensive toxicity assessments. Additional studies are needed to clarify the mechanisms of action of the encapsulated compounds; verify their systemic bioavailability, degree of homogenization of EO blends in poultry feed, and encapsulation efficiency; and evaluate their safety across a range of physiological conditions. Future research should also address the stability of the microcapsules during feed processing and storage, as well as their scalability and cost-effectiveness for commercial applications. Despite these limitations, the present findings remain robust and support the conclusion that lipid-encapsulated EO blends — when administered at appropriate doses — represent a promising, natural, and effective alternative to conventional antimicrobials for *Salmonella* control in poultry production systems.

4. Materials and Methods

4.1. Chemical Reagents

Mueller–Hinton Broth (MHB) was purchased from Oxoid (Hampshire, UK), and Brilliant Green Agar (BGA) from Kasvi (Spain). Selenite broth was obtained from

HiMedia (Mumbai, India), and Luria–Bertani (LB) broth from Sigma-Aldrich (St. Louis, MO, USA). Analytical standards, including carvacrol (W224502), thymol (T0501), cinnamaldehyde (C80687), and eugenol (E51791), were also obtained from Sigma-Aldrich (St. Louis, MO, USA). Phosphate-buffered saline (PBS, pH 7.2) was prepared using analytical-grade reagents.

4.2. Essential Oils

Sixteen EOs were purchased from Destilaria Bauru® (São Paulo, Brazil), and detailed specifications from each oil, such as origin and plant species, are arranged in Table 4 (extracted by steam distillation). For characterization, 1 mL aliquots of each EO were placed into individual Eppendorf tubes and weighed to determine their densities (mg/mL). EO aliquots (1 mL each) were individually placed into Eppendorf tubes, weighed to determine their densities (mg/mL), and stored under refrigeration (<5 °C) until the preparation of stock solutions. Stock solutions were initially prepared at a 1% EO (*v*/*v*) concentration, containing 1% Tween 80 as an emulsifier, with PBS (pH 7.2) completing the final volume; for EOs lacking antimicrobial activity at 1%, the concentrations were increased up to 10% EO. Finally, the stock solution was homogenized using a vortex mixer at 3800 rpm for five minutes to promote oil emulsification.

Table 4. Characteristics of each essential oil tested in vitro for antimicrobial activity against *Salmonella* Enteritidis.

Common name	Scientific name	Botanical source	Geographical origin
Oregano	*Origanum vulgare*	Leaf	Spain
Cinnamon	*Cinnamomum zeylanicum*	Bark	Brazil
Clove	*Eugenia caryophyllus*	Flower	Brazil
Mentha	*Mentha arvensis*	Leaf	Brazil
White thyme	*Thymus vulgaris*	Leaf	India
Citronella	*Cymbopogon nardus*	Leaf	Brazil
Lemongrass	*Cymbopogon flexuosos*	Leaf	Brazil
Rosemary	*Rosmarinus officinalis*	Leaf	Spain
Tea tree	*Melaleuca alternifolia*	Leaf	India

Black pepper	*Piper nigrum L.*	Fruit	India
Eucalyptus	*Eucalyptus globulus*	Leaf	Brazil
Ginger	*Zingiber officinale*	Root	China
Basil	*Ocimun basilicum L.*	Leaf	India
Copaiba	*Copaifera officinalis*	Leaf	Brazil
Sweet pear orange	*Citrus sinensis*	Fruit bark	Brazil
Bay laurel	*Laurus nobilis*	Leaf	Brazil

4.3. In Vitro Studies

4.3.1. Bacterial Strain

For this study, we selected an *S.* Enteritidis strain (SE P125 109) originally isolated from contaminated eggs and associated with human salmonellosis outbreaks in England. An overnight culture of *S*. Enteritidis was prepared by incubating 10 mL of MHB at 37 °C with orbital agitation at 150 rpm. After incubation, the bacterial suspension was diluted in phosphate-buffered saline (PBS, pH 7.2) and adjusted to approximately 10^{33} colony-forming units (CFU/mL) for use in vitro assays. All experimental procedures were carried out at the Department of Pathology, Reproduction and One Health (DPRSU), School of Agricultural and Veterinary Sciences, São Paulo State University (FCAV/Unesp).

4.3.2. Antimicrobial Activity

MIC was performed in sterile 96-well microplates following the Clinical and Laboratory Standards Institute guidelines [59], with minor adaptations based on Munive Nuñez et al. [60]. Briefly, 95 µL of MHB was added to each well, then 100 µL of the EO stock solution was added to the first well of each row, except for the positive control, which contained MHB and bacterial suspension only. Using a multichannel micropipette, we slowly homogenized the stock solution into the MHB. After homogenization, a two-fold serial dilution was performed by transferring 100 µL from the first to the subsequent wells. Next, 5 µL of the standardized bacterial suspension was added to all wells, except for the negative control (MHB + EO only), resulting in a final volume of 100 µL per well. Plates were incubated at 37 °C for 24 h. After incubation, 30 µL of 0.01% resazurin solution was added to each well, and results were obtained after 30 min, based on the visual reading of the colorimetric reaction occurrence, where blue indicated bacterial inhibition and pink indicated the absence of inhibition. The MIC was defined as the lowest EO concentration at which the well maintained a blue color.

For MBC, 10 µL measures from the last four wells showing no visible growth in the MIC assay were spot inoculated onto BGA plates, as described by Munive Nuñez et al. [60]. Plates were incubated at 37 °C for 24 h, and the MBC was defined as the lowest concentration at which no bacterial colonies were observed.

4.3.3. Synergism Assessment of Essential Oils

To evaluate the synergistic interactions between EOs, a Checkerboard assay was conducted using a 6 × 6 microplate format. Five EOs exhibiting the lowest MIC values against *S.* Enteritidis were selected, resulting in ten possible combinations. Stock solutions were individually prepared for each oil at sixteen times their respective MIC values (16 × MIC), mixed with 1% Tween 80, and adjusted to final volume with PBS.

For each EO combination, three 96-well microplates were prepared by initially dispensing 100 µL of MHB per well. On the first plate, 100 µL of EO from those exhibiting the lowest MIC values (in stock solution) was added into the first six wells of the initial column. After thorough homogenization, serial dilutions were performed horizontally by transferring 100 µL to subsequent columns. Similarly, on the second plate, 100 µL of another one from the lowest-MIC-value EO stock solution was added to the first six wells of the initial row, followed by vertical serial dilutions transferring 100 µL downward into subsequent rows. Afterwards, 50 µL from each of the first six columns of the first plate and 50 µL from each of the first six rows of the second plate were combined into corresponding wells on a third plate. This ensured that the highest concentrations of both oils were in the first well (top-left), progressively decreasing toward the lowest concentrations in the last well (bottom-right), as depicted in Figure 5. After combining these aliquots, 5 µL of bacterial suspension was added into each well, excluding the negative controls, maintaining identical conditions as previously described for MIC determination.

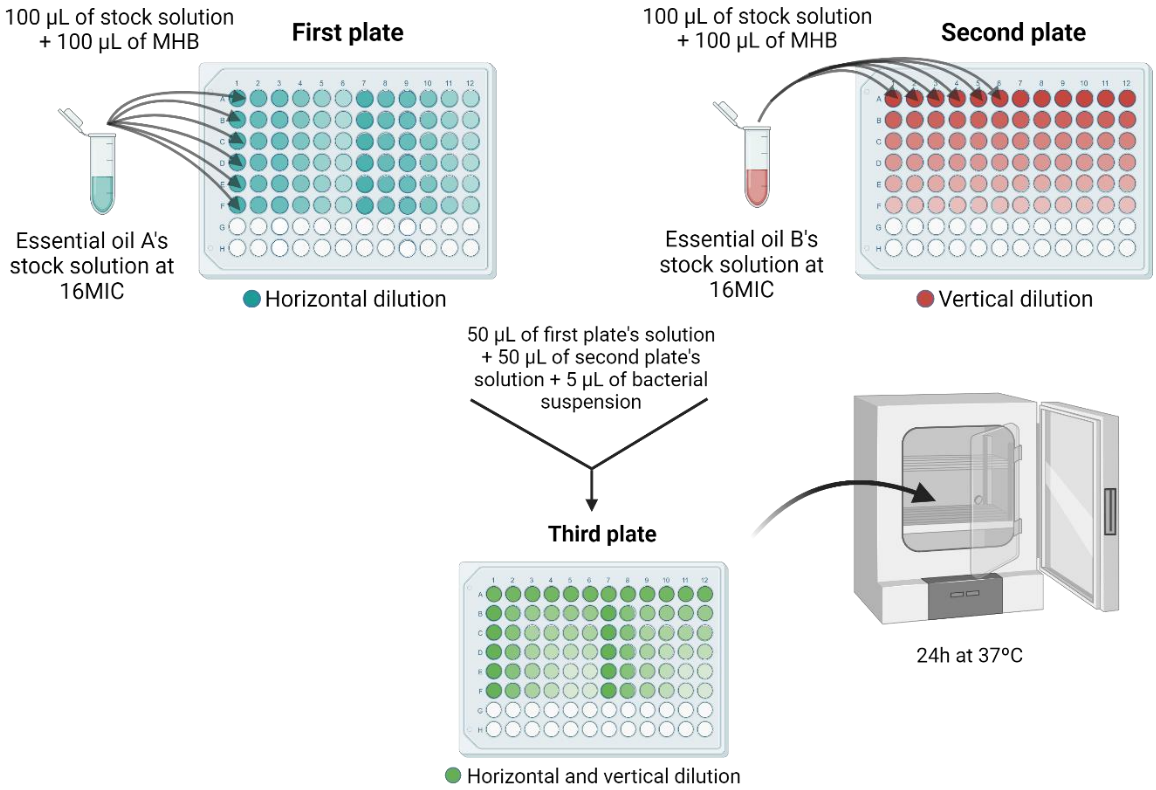


Figure 5. Method of the Checkboard assay. MHB = Mueller–Hinton Broth; MIC = minimum inhibitory concentration. This figure was created using Biorender.com.

To validate the EO stock solutions, MIC tests were repeated on separate plates using the same five oil solutions. Checkerboard and MIC assay plates were incubated at 37 °C for 24 h. Following incubation, 30 µL of 0.01% resazurin solution was added to each well to interpret visually bacterial growth inhibition, employing the same criteria as the standard MIC assay. The FICI Index was calculated using Equation (1), where "first EO" and "second EO" denote the MIC values for each EO in the mixture and individually. The calculated FICI values were interpreted as synergistic (*FICI* < 1), indifferent (*FICI* = 1), commutative (1 < *FICI* ≤ 2), or antagonistic (*FICI* > 2), according to Maggio et al. [15].

$$FICI = \frac{MIC^{A}mixture}{MIC^{A}alone} + \frac{MIC^{B}mixture}{MIC^{B}alone} \quad (1)$$

4.4. Encapsulation Strategy and Analytical Assessment

Of five EOs exhibiting the lowest MIC values, three of them were selected based on their favorable interaction profiles, with no antagonism observed in previous in vitro assessments. These oils were encapsulated within a hydrophobic matrix designed to prevent volatilization and promote the bioavailability of EOs. The formulation strategy considered physicochemical compatibility and additive behavior among components, guided by principles derived from the FICI.

EOs were encapsulated in collaboration with Agroceres Multimix Nutrição Animal Ltda., using hydrogenated vegetable fat as the encapsulation matrix. The process consisted of melting the fat, followed by the incorporation of EOs under continuous stirring until complete homogenization. The mixture was then cooled under controlled agitation, leading to the solidification of the lipid matrix. Agitation continued until a powdered product was obtained. Due to industrial confidentiality agreements, specific formulation details and processing conditions cannot be disclosed.

The particle size of the microcapsules was evaluated using optical microscopy (Olympus, DP73—Tokyo, Japan) with CellSens Standard© software, version 4.2 CS-ST-V4.2 (Olympus LS). Approximately 0.5 g of the encapsulated EO blend was deposited onto clean glass slides and imaged under a 20× objective lens. Two orthogonal diameters (length and width) were measured per capsule to account for possible asymmetry. Measurements were taken directly using the software and expressed in micrometers (µm). A total of 20 individual microcapsules were randomly selected and analyzed.

4.5. Analytical Performance

To evaluate the retention and stability of key active compounds, the encapsulated and non-encapsulated oils were analyzed in triplicate using chromatography (HPLC; Waters 2695 Separations Module), coupled with a photodiode array UV–Vis detector (Waters 2998, Photodiode Array Detector). Chromatographic separation was achieved using a C18 column (Agilent Technologies, 150 × 4.6 mm, 5 µm SB-C18) under isocratic conditions, with detection at 203 nm. The column oven was maintained at 45 °C, with a flow rate of 1.0 mL/min and an injection volume of 10 µL. The concentrations of active compounds in the EO blend were determined by integration of the chromatographic peak areas obtained via HPLC, similar to the method described by Wang et al. [61]. Quantification was carried out using external standards for carvacrol, thymol, eugenol, and cinnamaldehyde, with calibration curves prepared from standard solutions at known concentrations. For sample preparation, 103.5 mg of the EO blend was extracted in 25 mL of methanol in an ultrasonic bath for 30 min. A second dilution was performed by adding 0.10 mL of this extract to 10 mL of mobile phase (composed of 50% acetonitrile and 50% Milli-Q water), resulting in a final concentration of 41.4 µg/mL. The standards were prepared independently, with final concentrations of 9.9 µg/mL for carvacrol and thymol, 3.2 µg/mL for eugenol, and 10.5 µg/mL for cinnamaldehyde. Quantification was performed both before and after encapsulation.

4.6. Ethical Approval

The in vivo study was reviewed and approved by the Ethics Committee on Animal Experimentation (CEUA) of the School of Agricultural and Veterinary Sciences, São Paulo State University (FCAV/Unesp), under protocol number 2931/2024, on June 16, 2024.

4.6.1. Bacterial Inoculum

To prepare the inoculum, we used the same strain of *S.* Enteritidis (SE P125 109) as in the in vitro assay. This strain is resistant to nalidixic acid and spectinomycin ($SE^{NalSpcR}$). The strain was inoculated in LB broth and incubated at 37 °C for 24 h under constant agitation at 150 rpm. After incubation, the inoculum was diluted 10^3.

4.6.2. Experimental Design

For this study, 135 one-day-old broiler chicks were obtained from a commercial hatchery. These were randomly distributed into three groups (A, B, and C) of 45 animals each. Poultry in group A received a half dose of the EO blend (205 g/10 kg of feed), poultry in group B received the full dose of the EO blend (410 g/10 kg of feed), and poultry in group C received no treatment (the control group).

Upon arrival, sterile swabs were used to collect samples from the bottom of the transport boxes to confirm that the newly hatched chicks were free of *Salmonella* spp. The poultry chicks were housed in acclimatized rooms, separately in metal cages and provided with water and feed *ad libitum*, following species and lineage-specific recommendations. Poultry treated with the EO blend received the product mixed with feed throughout the experiment. To this end, the EO blend was incorporated into the feed and manually homogenized for approximately ten minutes.

On the second day of age, chicks were inoculated into the crop using oral gavage needles with 0.2 mL *S.* Enteritidis^{NalSpcR} culture containing approximately 1 × 10^6 CFU. Twice weekly after the challenge, cloacal swabs were collected from 15 broilers per group to inspect the excretion of *S.* Enteritidis^{NalSpcR}. Then, the swabs were placed in tubes containing 3 mL of selenite broth plus 0.4% novobiocin. Samples were then streaked onto BGA plates supplemented with nalidixic acid and spectinomycin (BGA$^{Nal/Spc}$). The plates and swab-containing tubes were incubated at 37 °C for 24 h.

To assess cecal colonization and systemic infection, five broilers per group were euthanized at 2-, 5-, 7-, 14-, and 21-day post-infection (dpi) for *S.* Enteritidis^{NalSpcR} enumeration in the liver, spleen, and ceca. Bacterial enumeration was carried out according to the methodology adopted by Berchieri Junior et al. [62]. The obtained CFU/mL values were converted to Log_{10} for statistical analysis and result interpretation.

To measure daily feed intake, the residual feed volume in the feeders was weighed daily and subtracted from the amount added the previous day at standardized times, ensuring a 24 h evaluation period. Data from all three groups were recorded daily in spreadsheets from the beginning to the end of the experiment.

4.6.3. Statistical Analysis

Statistical analysis was conducted using GraphPad Prism© software, version 10.2.3 for Windows©. Fecal shedding data from the three groups were plotted as positive and negative swabs and compared using the Chi-square test. Log-transformed bacterial counts in the liver, spleen, and ceca were analyzed using a two-way ANOVA, followed by Bonferroni's multiple comparison test. Data on daily feed intake were analyzed using one-way ANOVA and compared using the Chi-square test. Statistical significance was admitted when the *p*-value was lower than 5% ($p < 0.05$).

5. Conclusions

This study provides important insights into the in vitro and in vivo antimicrobial activity of various EOs against *S.* Enteritidis. Our results indicate that microencapsulated cinnamon, clove, and oregano EOs are an effective natural alternative for reducing the fecal excretion of *S.* Enteritidis and its systemic infection in broilers, without affecting the daily feed intake of treated broilers. These findings strongly contribute to the maintenance of the One Health approach and minimizing the emergence of resistant or multidrug-resistant bacteria originating from animal production. We emphasize the need for further research to determine optimal and potentially toxic concentrations for poultry, including the assessment of the effects of these phytobiotics on the gut microbiota and tissue integrity in broiler chickens. Therefore, further research exploring lower concentrations and their effects on infection and poultry's health is needed to establish optimal therapeutic dosing standards in broiler chickens.

Author Contribuitions

Conceptualization, H.L.d.S.R., A.B.J., M.M.S.S. and V.P.B.; methodology, H.L.d.S.R., A.B.J. and C.A.R.-B.; validation, H.L.d.S.R., I.M.M.K., V.P.B., A.L.C.R., V.A.F., M.F.F.D. and A.M.A.; formal analysis, H.L.d.S.R., I.M.M.K., N.P.D.B.G.S. and T.A.B.; investigation, H.L.d.S.R., I.M.M.K., V.P.B., A.L.C.R., V.A.F., M.F.F.D. and A.M.A.; resources, C.A.R.-B., T.A.B. and N.P.D.B.G.S.; data curation, H.L.d.S.R., I.M.M.K., V.P.B., A.L.C.R., V.A.F., M.F.F.D. and A.M.A.; writing—original draft preparation, H.L.d.S.R., V.P.B. and C.A.R.-B.; writing—review and editing, A.B.J., C.A.R.-B., V.P.B. and M.M.S.S.; supervision, A.B.J., C.A.R.-B. and V.P.B. All authors have read and agreed to the published version of the manuscript.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001, the Fundação de Amparo à Pesquisa do Estado de São Paulo—Brasil (FAPESP) (#2024/03279-0), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico—Brasil (CNPq) (Grant Number 175342/2023-7 (Valdinete Pereira Benevides)).

Institutional Review Board Statement

This study was conducted in accordance with current national and international guidelines for the use of animals in research, and with full respect for the welfare of the species involved. Moreover, the study was reviewed and approved by the Ethics

Committee on Animal Experimentation (CEUA) of the School of Agricultural and Veterinary Sciences, São Paulo State University (FCAV/Unesp), under protocol number 2931/2024, on 16 June 2024.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data are contained within the article and **Supplementary Materials**.

Acknowledgements

We acknowledge the School of Agricultural and Veterinary Sciences (FCAV/Unesp), Agroceres Multimix Nutrição Animal Ltda., and the team at the Laboratory of Avian Pathology for their valuable contributions.

Conflicts of Interest

Nayla Pádua Del Bianco Gontijo Souki and Tarley Araújo Barros are affiliated with Agroceres Multimix Animal Nutrition, Rio Claro, Brazil, and declare a potential conflict of interest due to their employment. All other authors declare no conflicts of interest.

Abbreviations

The following abbreviations have been used in this article:

SE	*Salmonella enterica* subsp. *enterica* serotype Enteritidis
EOs	Essential oils
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
FICI	Fractional Inhibitory Concentration Index
CEUA	Ethics Committee on Animal Experimentation
MHB	Mueller–Hinton Broth

LBB	Luria–Bertani Broth
BGA	Brilliant Green Agar
Nal/Spc	Nalidixic acid/Spectinomycin
CFU	Colony-Forming Unit
v/*v*	Volume by volume
HPLC	High-Performance Liquid Chromatography

References

1. Li, S.; He, Y.; Mann, D.A.; Deng, X. Global Spread of *Salmonella* Enteritidis via Centralized Sourcing and International Trade of Poultry Breeding Stocks. *Nat. Commun.* **2021**, *12*, 5109.
2. Velge, P.; Menanteau, P.; Chaumeil, T.; Barilleau, E.; Trotereau, J.; Virlogeux-Payant, I. Two In Vivo Models to Study *Salmonella* Asymptomatic State in Chicks. In *Bacterial Virulence*; Methods in Molecular Biology; Humana: New York, NY, USA, 2022; ISBN 9781071619704.
3. de Mesquita Souza Saraiva, M.; Lim, K.; do Monte, D.F.M.; Givisiez, P.E.N.; Alves, L.B.R.; de Freitas Neto, O.C.; Kariuki, S.; Júnior, A.B.; de Oliveira, C.J.B.; Gebreyes, W.A. Antimicrobial Resistance in the Globalized Food Chain: A One Health Perspective Applied to the Poultry Industry. *Braz. J. Microbiol.* **2022**, *53*, 465–486.
4. Saraiva, M.M.S.; Moreira Filho, A.L.B.; Freitas Neto, O.C.; Silva, N.M.V.; Givisiez, P.E.N.; Gebreyes, W.A.; Oliveira, C.J.B. Off-Label Use of Ceftiofur in One-Day Chicks Triggers a Short-Term Increase of ESBL-Producing E. Coli in the Gut. *PLoS ONE* **2018**, *13*, e0203158.
5. Roque-Borda, C.A.; Primo, L.M.D.G.; Medina-Alarcón, K.P.; Campos, I.C.; Nascimento, C.F.; Saraiva, M.M.S.; Berchieri Junior, A.; Fusco-Almeida, A.M.; Mendes-Giannini, M.J.S.; Perdigão, J.; et al. Antimicrobial Peptides: A Promising Alternative to Conventional Antimicrobials for Combating Polymicrobial Biofilms. *Adv. Sci.* **2024**, *12*, e2410893.

6. Puvača, N.; Milenković, J.; Galonja Coghill, T.; Bursić, V.; Petrović, A.; Tanasković, S.; Pelić, M.; Ljubojević Pelić, D.; Miljković, T. Antimicrobial Activity of Selected Essential Oils against Selected Pathogenic Bacteria: In Vitro Study. *Antibiotics* **2021**, *10*, 546.
7. Huerta Lorenzo, B.; Galán-Relaño, Á.; Barba-Sánchez, E.; Romero-Salmoral, A.; Solarte Portilla, A.L.; Gómez-Gascón, L.; Astorga Márquez, R.J. Potentiation of the Antimicrobial Effect of Oxytetracycline Combined with Cinnamon, Clove, Oregano, and Red Thyme Essential Oils against MDR *Salmonella enterica* Strains. *Animals* **2024**, *14*, 1347.
8. Robinson, K.; Assumpcao, A.L.F.V.; Arsi, K.; Donoghue, A.; Jesudhasan, P.R.R. Ability of Garlic and Ginger Oil to Reduce *Salmonella* in Post-Harvest Poultry. *Animals* **2022**, *12*, 2974.
9. Islam, Z.; Sultan, A.; Khan, S.; Khan, K.; Jan, A.U.; Aziz, T.; Alharbi, M.; Alshammari, A.; Alasmari, A.F. Effects of an Organic Acids Blend and Coated Essential Oils on Broiler Growth Performance, Blood Biochemical Profile, Gut Health, and Nutrient Digestibility. *Ital. J. Anim. Sci.* **2024**, *23*, 152–163.
10. Galgano, M.; Pellegrini, F.; Fracchiolla, G.; Mrenoshki, D.; Zarea, A.A.K.; Bianco, A.; Del Sambro, L.; Capozzi, L.; Schiavone, A.; Saleh, M.S.; et al. Pilot Study on the Action of Thymus Vulgaris Essential Oil in Treating the Most Common Bacterial Contaminants and *Salmonella enterica* Subsp. *enterica* Serovar Derby in Poultry Litter. *Antibiotics* **2023**, *12*, 436.
11. Oliveira, G.D.S.; McManus, C.; de Araújo, M.V.; de Sousa, D.E.R.; de Macêdo, I.L.; Castro, M.B.; Santos, V.M.D. Sanitizing Hatching Eggs with Essential Oils: Avian and Microbiological Safety. *Microorganisms* **2023**, *11*, 1890.
12. Hu, Z.; Liu, L.; Guo, F.; Huang, J.; Qiao, J.; Bi, R.; Huang, J.; Zhang, K.; Guo, Y.; Wang, Z. Dietary Supplemental Coated Essential Oils and Organic Acids Mixture Improves Growth Performance and Gut Health along with Reduces *Salmonella* Load of Broiler Chickens Infected with *Salmonella* Enteritidis. *J. Anim. Sci. Biotechnol.* **2023**, *14*, 95.
13. Li, Z.; Li, T.; Tang, J.; Huang, L.; Ding, Y.; Zeng, Z.; Liu, J. Antibacterial Activity of Surfactin and Synergistic Effect with Conventional Antibiotics Against Methicillin-

Resistant *Staphylococcus aureus* Isolated from Patients with Diabetic Foot Ulcers. *Diabetes Metab. Syndr. Obes.* **2023**, *16*, 3727–3737.

14. Wang, L.; Fang, J.; Wang, H.; Zhang, B.; Wang, N.; Yao, X.; Li, H.; Qiu, J.; Deng, X.; Leng, B.; et al. Natural Medicine Can Substitute Antibiotics in Animal Husbandry: Protective Effects and Mechanisms of Rosewood Essential Oil against *Salmonella* Infection. *Chin. J. Nat. Med.* **2024**, *22*, 785–796.
15. Maggio, F.; Lauteri, C.; Rossi, C.; Ferri, G.; Serio, A.; Vergara, A.; Paparella, A. Combined Effect of Tetracycline Compounds and Essential Oils on Antimicrobial Resistant *Salmonella enterica* Isolated from the Swine Food Chain. *Front. Microbiol.* **2024**, *15*, 1439286.
16. Rodrigues, H.L.S.; Kolososki, I.M.M.; Benevides, V.P.; Saraiva, M.M.S.; Berchieri, A.; Junior, A.B. Essential Oils Used in the Poultry Industry: Would It Be an Effective Green Alternative against *Salmonella* Spp. Dissemination and Antimicrobial Resistance? *Microbe* **2025**, *6*, 100248.
17. Mo, K.; Li, J.; Liu, F.; Xu, Y.; Huang, X.; Ni, H. Superiority of Microencapsulated Essential Oils Compared With Common Essential Oils and Antibiotics: Effects on the Intestinal Health and Gut Microbiota of Weaning Piglet. *Front. Nutr.* **2022**, *8*, 808106.
18. Roque-Borda, C.A.; Chávez-Morán, M.R.; Primo, L.M.D.G.; Márquez Montesinos, J.C.E.; Borges Cardoso, V.M.; Saraiva, M.M.S.; Marcos, C.M.; Chorilli, M.; Albericio, F.; de la Torre, B.G.; et al. Alginate-Pectin Microparticles Embedding Self-Assembling Antimicrobial Peptides and Resveratrol for Antimicrobial and Anti-Inflammatory Applications. *Food Hydrocoll.* **2025**, *167*, 111454.
19. Saporito, F.; Sandri, G.; Bonferoni, M.C.; Rossi, S.; Boselli, C.; Icaro Cornaglia, A.; Mannucci, B.; Grisoli, P.; Vigani, B.; Ferrari, F. Essential Oil-Loaded Lipid Nanoparticles for Wound Healing. *Int. J. Nanomed.* **2017**, *13*, 175–186.
20. Silva, E.F.D.; Santos, F.A.L.D.; Pires, H.M.; Bastos, L.M.; Ribeiro, L.N.M. Lipid Nanoparticles Carrying Essential Oils for Multiple Applications as Antimicrobials. *Pharmaceutics* **2025**, *17*, 178.
21. Lai, F.; Wissing, S.A.; Müller, R.H.; Fadda, A.M. Artemisia Arborescens L Essential Oil-Loaded Solid Lipid Nanoparticles for Potential Agricultural

Application: Preparation and Characterization. *AAPS PharmSciTech* **2006**, *7*, E10–E18.

22. Katopodi, A.; Detsi, A. Solid Lipid Nanoparticles and Nanostructured Lipid Carriers of Natural Products as Promising Systems for Their Bioactivity Enhancement: The Case of Essential Oils and Flavonoids. *Colloids Surf. A Physicochem. Eng. Asp.* **2021**, *630*, 127529.
23. Shetta, A.; Kegere, J.; Mamdouh, W. Comparative Study of Encapsulated Peppermint and Green Tea Essential Oils in Chitosan Nanoparticles: Encapsulation, Thermal Stability, in-Vitro Release, Antioxidant and Antibacterial Activities. *Int. J. Biol. Macromol.* **2019**, *126*, 731–742.
24. Granata, G.; Stracquadanio, S.; Leonardi, M.; Napoli, E.; Malandrino, G.; Cafiso, V.; Stefani, S.; Geraci, C. Oregano and Thyme Essential Oils Encapsulated in Chitosan Nanoparticles as Effective Antimicrobial Agents against Foodborne Pathogens. *Molecules* **2021**, *26*, 4055.
25. de Carvalho, S.Y.B.; Almeida, R.R.; Pinto, N.A.R.; de Mayrinck, C.; Vieira, S.S.; Haddad, J.F.; Leitão, A.A.; de L. Guimarães, L.G. Encapsulation of Essential Oils Using Cinnamic Acid Grafted Chitosan Nanogel: Preparation, Characterization and Antifungal Activity. *Int. J. Biol. Macromol.* **2021**, *166*, 902–912.
26. Li, Y.-x.; Erhunmwunsee, F.; Liu, M.; Yang, K.; Zheng, W.; Tian, J. Antimicrobial Mechanisms of Spice Essential Oils and Application in Food Industry. *Food Chem.* **2022**, *382*, 132312.
27. Omonijo, F.A.; Ni, L.; Gong, J.; Wang, Q.; Lahaye, L.; Yang, C. Essential Oils as Alternatives to Antibiotics in Swine Production. *Anim. Nutr.* **2018**, *4*, 126–136.
28. Bona, T.D.M.M.; Pickler, L.; Miglino, L.B.; Kuritza, L.K.; Vasconcelos, S.P.; Santin, E. Óleo Essencial de Orégano, Alecrim, Canela e Extrato de Pimenta No Controle de *Salmonella*, *Eimeria* e *Clostridium* Em Frangos de Corte. *Pesqui. Veterinária Bras.* **2012**, *32*, 411–418.
29. Guedes Frazzon, A.P.; Saldanha, M.; Junior, L.C.; Frazzon, J. Modulation of Gene Expression by Essential Oils in Bacteria. *Adv. Biotechnol. Microbiol.* **2018**, *8*, 555731.
30. Spisni, E.; Petrocelli, G.; Imbesi, V.; Spigarelli, R.; Azzinnari, D.; Donati Sarti, M.; Campieri, M.; Valerii, M.C. Antioxidant, Anti-Inflammatory, and Microbial-

Modulating Activities of Essential Oils: Implications in Colonic Pathophysiology. *Int. J. Mol. Sci.* **2020**, *21*, 4152.

31. Nikolić, M.; Stojković, D.; Glamočlija, J.; Ćirić, A.; Marković, T.; Smiljković, M.; Soković, M. Could Essential Oils of Green. and Black Pepper Be Used as Food Preservatives? *J. Food Sci. Technol.* **2015**, *52*, 6565–6573.
32. Tariq, S.; Wani, S.; Rasool, W.; Shafi, K.; Bhat, M.A.; Prabhakar, A.; Shalla, A.H.; Rather, M.A. A Comprehensive Review of the Antibacterial, Antifungal and Antiviral Potential of Essential Oils and Their Chemical Constituents against Drug-Resistant Microbial Pathogens. *Microb. Pathog.* **2019**, *134*, 103580.
33. Alibi, S.; Selma, W.B.; Mansour, H.B.; Navas, J. Activity of Essential Oils Against Multidrug-Resistant *Salmonella* Enteritidis. *Curr. Microbiol.* **2022**, *79*, 273.
34. Somrani, M.; Huertas, J.P.; Iguaz, A.; Debbabi, H.; Palop, A. Biofilm Busters: Exploring the Antimicrobial and Antibiofilm Properties of Essential Oils against *Salmonella* Enteritidis. *Food Sci. Technol. Int.* **2024**.
35. Purkait, S.; Bhattacharya, A.; Bag, A.; Chattopadhyay, R.R. Synergistic Antibacterial, Antifungal and Antioxidant Efficacy of Cinnamon and Clove Essential Oils in Combination. *Arch. Microbiol.* **2020**, *202*, 1439–1448.
36. Goñi, P.; López, P.; Sánchez, C.; Gómez-Lus, R.; Becerril, R.; Nerín, C. Antimicrobial Activity in the Vapour Phase of a Combination of Cinnamon and Clove Essential Oils. *Food Chem.* **2009**, *116*, 982–989.
37. Bassolé, I.H.N.; Juliani, H.R. Essential Oils in Combination and Their Antimicrobial Properties. *Molecules* **2012**, *17*, 3989–4006.
38. Roque-Borda, C.A.; Souza Saraiva, M.M.; Monte, D.F.M.; Rodrigues Alves, L.B.; de Almeida, A.M.; Ferreira, T.S.; de Lima, T.S.; Benevides, V.P.; Cabrera, J.M.; Claire, S.; et al. HPMCAS-Coated Alginate Microparticles Loaded with Ctx(Ile 21)-Ha as a Promising Antimicrobial Agent against Salmonella Enteritidis in a Chicken Infection Model. *ACS Infect. Dis.* **2022**, *8*, 472–481.
39. Roque-Borda, C.A.; Saraiva, M.M.S.; Macedo, W.D., Jr.; Márquez Montesinos, J.C.E.; Meneguin, A.B.; Toledo Borges, A.B.; Crusca, E., Jr.; Garrido, S.S.; de Almeida, A.M.; Marchetto, R.; et al. Chitosan and HPMCAS Double-Coating as Protective Systems for Alginate Microparticles Loaded with Ctx(Ile21)-Ha

Antimicrobial Peptide to Prevent Intestinal Infections. *Biomaterials* **2023**, *293*, 121978.

40. Hasanzadeh Baboli, N.; Hosseini, S.F.; Gharsallaoui, A. Antibacterial and Anti-biofilm Properties of Cinnamaldehyde-loaded Nanoliposomes against *Listeria monocytogenes* and *Salmonella enteritidis* Adhered to Stainless Steel. *Int. J. Food Sci. Technol.* **2023**, *58*, 5275–5282.
41. Yucel, C.; Quagliariello, V.; Iaffaioli, R.V.; Ferrari, G.; Donsì, F. Submicron Complex Lipid Carriers for Curcumin Delivery to Intestinal Epithelial Cells: Effect of Different Emulsifiers on Bioaccessibility and Cell Uptake. *Int. J. Pharm.* **2015**, *494*, 357–369.
42. Ramalingam, S.; Sahu, B.; Rao, J.R. Hybrid Nanoparticles Emulsified Vegetable Oil as an Environmentally Friendly and Sustainable Leather Fatliquoring Agent. *Process Saf. Environ. Prot.* **2023**, *179*, 896–906.
43. Jurić, S.; Jurić, M.; Siddique, M.A.B.; Fathi, M. Vegetable Oils Rich in Polyunsaturated Fatty Acids: Nanoencapsulation Methods and Stability Enhancement. *Food Rev. Int.* **2022**, *38*, 32–69.
44. Moharreri, M.; Vakili, R.; Oskoueian, E.; Rajabzadeh, G. Effects of Microencapsulated Essential Oils on Growth Performance and Biomarkers of Inflammation in Broiler Chickens Challenged with *Salmonella* Enteritidis. *J. Saudi Soc. Agric. Sci.* **2022**, *21*, 349–357.
45. Salam, H.S.H.; Mohammed, A.N.; Hosni, A.R.; Shehata, A.A.E. Tracking of Resistant *Salmonella* Species in Poultry Farms: New Method of Control Using Essential Oils Nano-Emulsion Conjugated with Antimicrobial Agents. *Trop. Anim. Sci. J.* **2021**, *44*, 489–501.
46. Qu, J.; Zuo, X.; Xu, Q.; Li, M.; Zou, L.; Tao, R.; Liu, X.; Wang, X.; Wang, J.; Wen, L.; et al. Effect of Two Particle Sizes of Nano Zinc Oxide on Growth Performance, Immune Function, Digestive Tract Morphology, and Intestinal Microbiota Composition in Broilers. *Animals* **2023**, *13*, 1454.
47. El-Shall, N.A.; Shewita, R.S.; Abd El-Hack, M.E.; AlKahtane, A.; Alarifi, S.; Alkahtani, S.; Abdel-Daim, M.M.; Sedeik, M.E. Effect of Essential Oils on the Immune Response to Some Viral Vaccines in Broiler Chickens, with Special Reference to Newcastle Disease Virus. *Poult. Sci.* **2020**, *99*, 2944–2954.

48. Hesabi Nameghi, A.; Edalatian, O.; Bakhshalinejad, R. Effects of a Blend of Thyme, Peppermint and Eucalyptus Essential Oils on Growth Performance, Serum Lipid and Hepatic Enzyme Indices, Immune Response and Ileal Morphology and Microflora in Broilers. *J. Anim. Physiol. Anim. Nutr.* **2019**, *103*, 1388–1398.
49. Cerisuelo, A.; Marín, C.; Sánchez-Vizcaíno, F.; Gómez, E.A.; De La Fuente, J.M.; Durán, R.; Fernández, C. The Impact of a Specific Blend of Essential Oil Components and Sodium Butyrate in Feed on Growth Performance and *Salmonella* Counts in Experimentally Challenged Broilers. *Poult. Sci.* **2014**, *93*, 599–606.
50. Olgun, O.; Yıldız, A.Ö. Effect of Dietary Supplementation of Essential Oils Mixture on Performance, Eggshell Quality, Hatchability, and Mineral Excretion in Quail Breeders. *Environ. Sci. Pollut. Res.* **2014**, *21*, 13434–13439.
51. Stingelin, G.M.; Scherer, R.S.; Machado, A.C.; Piva, A.; Grilli, E.; Penha Filho, R.C. The Use of Thymol, Carvacrol and Sorbic Acid in Microencapsules to Control *Salmonella* Heidelberg, *S.* Minnesota and *S.* Typhimurium in Broilers. *Front. Vet. Sci.* **2023**, *9*, 1046395.
52. Laptev, G.Y.; Yildirim, E.A.; Ilina, L.A.; Filippova, V.A.; Kochish, I.I.; Gorfunkel, E.P.; Dubrovin, A.V.; Brazhnik, E.A.; Narushin, V.G.; Novikova, N.I.; et al. Effects of Essential Oils-Based Supplement and *Salmonella* Infection on Gene Expression, Blood Parameters, Cecal Microbiome, and Egg Production in Laying Hens. *Animals* **2021**, *11*, 360.
53. Rojas-Armas, J.P.; Arroyo-Acevedo, J.L.; Ortiz-Sánchez, J.M.; Palomino-Pacheco, M.; Hilario-Vargas, H.J.; Herrera-Calderón, O.; Hilario-Vargas, J. Potential Toxicity of the Essential Oil from *Minthostachys Mollis*: A Medicinal Plant Commonly Used in the Traditional Andean Medicine in Peru. *J. Toxicol.* **2019**, *2019*, 1987935.
54. Gebremickael, A.; Gebru, G.; Debella, A.; Addis, G. Acute and Sub-Chronic Oral Toxicity Evaluation of Eucalyptus Globulus Essential Oil-Water Emulsion in Mice. *J. Cytol. Histol.* **2017**, *8*.
55. Wojtunik-Kulesza, K.A. Toxicity of Selected Monoterpenes and Essential Oils Rich in These Compounds. *Molecules* **2022**, *27*, 1716.

56. Hu, Y.; He, Z.; Zhang, J.; Zhang, C.; Wang, Y.; Zhang, W.; Zhang, F.; Zhang, W.; Gu, F.; Hu, W. Effect of Piper Nigrum Essential Oil in Dextran Sulfate Sodium (DSS)-Induced Colitis and Its Potential Mechanisms. *Phytomedicine* **2023**, *119*, 155024.
57. Dheyauldeen Salahdin, O.; Othman, H.; Hafsan, H.; Mohammed, F.; Ahmed Hamza, T.; Kadhim, M.M.; Aravindhan, S.; Prakaash, A.S.; Fakri Mustafa, Y. Effect of Licorice Essential Oil (Glycyrrhizaglabraglabra) on Performance and Some Biochemical Parameters of Broiler Chickens. *Arch. Razi Inst.* **2023**, *78*, 89–99.
58. Huang, J.; Guo, F.; Abbas, W.; Hu, Z.; Liu, L.; Qiao, J.; Bi, R.; Xu, T.; Zhang, K.; Huang, J.; et al. Effects of Microencapsulated Essential Oils and Organic Acids Preparation on Growth Performance, Slaughter Characteristics, Nutrient Digestibility and Intestinal Microenvironment of Broiler Chickens. *Poult. Sci.* **2024**, *103*, 103655.
59. Clinical and Laboratory Standards Institute. *CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing*, 28th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018; Volume 1, ISBN 156238838X.
60. Munive Nuñez, K.V.; da S. Abreu, A.C.; de Almeida, J.M.; Gonçalves, J.L.; Bonsaglia, É.C.R.; dos Santos, M.V.; Silva, N.C.C. Antimicrobial Activity of Selected Essential Oils against *Staphylococcus Aureus* from Bovine Mastitis. *Dairy* **2024**, *5*, 54–65.
61. Wang, P.; Chi, J.; Guo, H.; Wang, S.-X.; Wang, J.; Xu, E.-P.; Dai, L.-P.; Wang, Z.-M. Identification of Differential Compositions of Aqueous Extracts of *Cinnamomi Ramulus* and *Cinnamomi Cortex*. *Molecules* **2023**, *28*, 2015.
62. Berchieri, A.J.; Wigley, P.; Page, K.; Murphy, C.K.; Barrow, P.A. Further Studies on Vertical Transmission and Persistence of *Salmonella enterica* Serovar Enteritidis Phage Type 4 in Chickens. *Avian Pathol.* **2001**, *30*, 297–310.

CAPÍTULO 4 – Considerações finais

Os estudos recentemente publicados (Capítulos 2 e 3) contribuem de maneira substancial para o avanço do conhecimento sobre o uso de OEs na avicultura, especialmente no controle de SE em frangos de corte. Os resultados experimentais demonstram que o *blend* microencapsulado contendo OEs de canela, orégano e cravo é capaz de reduzir significativamente tanto a excreção fecal de SE quanto a colonização cecal, atingindo reduções de aproximadamente 4 $\log_{10}$, sem impactos adversos sobre o consumo de ração. Observou-se ainda que metade da dose promoveu o melhor desempenho antimicrobiano, indicando que a resposta biológica aos OEs pode não ser linear e reforçando a necessidade de estabelecer dosagens terapêuticas ideais.

A revisão sistemática evidencia vantagens relevantes da microencapsulação, incluindo maior estabilidade dos compostos bioativos, a proteção contra a degradação precoce, a liberação gradual ao longo do trato gastrointestinal e a melhor biodisponibilidade. Por outro lado, são destacadas as limitações atuais da literatura, entre elas o número ainda reduzido de estudos *in vivo* direcionados ao controle de *Salmonella* spp. em aves a partir de OEs, bem como a escassez de dados sobre a faixa entre doses eficazes e limiares potencialmente tóxicos para a espécie avícola. Além disso, questões relacionadas ao custo de produção e à viabilidade econômica das formulações microencapsuladas permanecem pouco exploradas e podem representar um desafio para sua adoção comercial, sobretudo se a eficácia depender de concentrações próximas ao limite de segurança.

Sob a perspectiva *One Health*, os OEs microencapsulados apresentam como uma alternativa natural aos antimicrobianos convencionais, contribuindo para a redução da carga de SE na produção avícola e, simultaneamente, para a diminuição da pressão seletiva que favorece à resistência aos antimicrobianos. Todavia, para consolidar essa estratégia como ferramenta aplicável em escala industrial, são necessários estudos adicionais que abordem a farmacocinética dos compostos, seus mecanismos de ação, as interações com a microbiota intestinal e os efeitos sobre parâmetros produtivos, além de avaliações de segurança em longo prazo.

Em síntese, os trabalhos depositados aqui indicam que o uso de OEs microencapsulados representa uma abordagem promissora para o controle de *S.* Enteritidis em frangos de corte, apresentando eficácia significativa e ausência de efeitos adversos no consumo alimentar de frangos. Esses resultados contribuem para o avanço de práticas sustentáveis na produção animal e se alinham com as metas estabelecidas pela Organização das Nações Unidas (ONU).

REFERÊNCIAS

Abou-Jaoudeh C, Andary J, Abou-Khalil R (2024) Antibiotic residues in poultry products and bacterial resistance: A review in developing countries. **J. Infect. Public Health** 17(12):102592. doi.org/10.1016/j.jiph.2024.102592

Alibi S, Selma WB, Mansour HB, Navas J (2022) Activity of essential oils against multidrug-resistant *Salmonella* Enteritidis. **Current Microbiology** 79:273. doi.org/10.1007/s00284-022-02938-x

Aljuwayd M, Malli IA, Kwon YM (2023) Application of eugenol in poultry to control *Salmonella* colonization and spread. **Veterinary Sciences** 10(2). doi.org/10.3390/vetsci10020151

Amiri N, Afsharmanesh M, Salarmoini A, et al. (2021) Nanoencapsulation (*in vitro* and *in vivo*) as an efficient technology to boost the potential of garlic essential oil as an alternative to antibiotics in broiler nutrition. **Animal** 15(1):100022. doi.org/10.1016/j.animal.2020.100022

Barrow PA, Huggins MB, Lovell MA, et al. (1987) Observations on the pathogenesis of experimental *Salmonella* Typhimurium infection in chickens. **Research in Veterinary Science** 42:194–199. doi.org/10.1016/S0034-5288(18)30685-4

Bernardos A, Marina T, Zacek P, et al. (2014) Antifungal effect of essential oil components against *Aspergillus niger* when loaded into silica mesoporous supports. **J. Sci. Food Agric.** 95(14). doi.org/10.1002/jsfa.7022

Berchieri Jr A, Wigley PK, Page RK, et al. (2001) Further studies on vertical transmission and persistence of *Salmonella enterica* serovar Enteritidis phage type 4 in chickens. **Avian Pathology** 30(4):297–310. doi.org/10.1080/03079450120066304

Bona TDM, Pickler L, Miglino LB, et al. (2012) Óleo essencial de orégano, alecrim, canela e extrato de pimenta no controle de *Salmonella*, *Eimeria* e *Clostridium* em frangos de corte. **Pesq. Vet. Bras.** 32(5). doi.org/10.1590/S0100-736X2012000500009

Cabral ISR, Oldoni TLC, Prado A, et al. (2009) Phenolic composition, antibacterial and antioxidant activities of Brazilian red propolis. **Química Nova** 32(6):1523–1527. doi.org/10.1590/S0100-40422009000600031

CLSI – Clinical and Laboratory Standards Institute (2018) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. 11ª ed.

D'Costa VM, King CE, Kalan L (2011) Antibiotic resistance is ancient. **Nature** 477:457–461. doi.org/10.1038/nature10388

Dittoe DK, Ricke SC, Kiess AS (2018) Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. **Front. Vet. Sci.** doi.org/10.3389/fvets.2018.00216

Enan G, Amen S, El-badiea AA, et al. (2023) The pathogen inhibition effects of probiotics and prebiotics against *Salmonella* spp. in chicken. **Ann. Anim. Sci.** 23(2). doi.org/10.2478/aoas-2022-0081

Ferreira TS, Ravetti R, Rubio MS, et al. (2022) Inclusion of organic acids in drinking water and feed for the control of *Salmonella* Heidelberg in broilers. **Braz. J. Poultry Sci.** 24(2). doi.org/10.1590/1806-9061-2020-1427

Freitas Neto OC, Filho RACP, Berchieri Jr A (2020) Salmoneloses aviárias. In: Andreatti Filho RL, Berchieri Jr A, Silva EN, *Doenças das Aves*. Campinas: FACTA, 3ª ed, p. 495–517.

Gadde U, Kim WH, Oh ST, et al. (2017) Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. **Animal Health Research Reviews** 18(1):26–45. doi.org/10.1017/S1466252316000207

Galgano M, Pellegrini F, Fracchiolla G, et al. (2023) Pilot study on the action of *Thymus vulgaris* essential oil in treating bacterial contaminants and *Salmonella* Derby in poultry litter. **Antibiotics** 12(3). doi.org/10.3390/antibiotics12030436

Guard-Petter J (2001) The chicken, the egg and *Salmonella Enteritidis*. **Environmental Microbiology** 3(7):421–430. doi.org/10.1046/j.1462-2920.2001.00213.x

Hu Z, Liu L, Guo F, et al. (2023) Dietary supplemental coated essential oils and organic acids mixture improves growth performance and gut health and reduces *Salmonella* Enteritidis load in broilers. **J. Anim. Sci. Biotechnol.** doi.org/10.1186/s40104-023-00889-2

Immerseel F, Russell JB, Flythe MD, et al. (2007) The use of organic acids to combat *Salmonella* in poultry: A mechanistic explanation. **Avian Pathology** 35(3):182–188. doi.org/10.1080/03079450600711045

Ibrahim D, Abdelfattah-Hassan A, Badawi M, et al. (2021) Thymol nanoemulsion promotes broiler growth, gut barrier and microbiota, and protects against *Salmonella* Typhimurium. **Scientific Reports** 11:7742. doi.org/10.1038/s41598-021-86990-w

Iwinski H, Wodz K, Chodkowska K, et al. (2022) *In vitro* evaluation of antimicrobial effect of phytobiotics mixture on *Salmonella* spp. isolated from broilers. **Antibiotics** 11(7). doi.org/10.3390/antibiotics11070868

Johny AK, Darre MJ, Donoghue AM, et al. (2010) Antibacterial effect of trans-cinnamaldehyde, eugenol, carvacrol and thymol on *Salmonella* Enteritidis and *Campylobacter jejuni* in chicken cecal contents. **J. Appl. Poult. Res.** 19:237–244. doi.org/10.3382/japr.2010-00181

Karabasanavar NS, Madhavaprasad CB, Gopalakrishna SA, et al. (2020) Prevalence of *Salmonella* Enteritidis and Typhimurium in poultry and poultry products. **J. Food Safety** 40(6). doi.org/10.1111/jfs.12852

Laudadio V, Passantino L, Perillo A, et al. (2012) Productive performance and intestinal mucosa histology of broilers fed different dietary protein levels. **Poultry Science** 91(1):265–270. doi.org/10.3382/ps.2011-01675

Liu Q, Huang H, Chen H, et al. (2019) Food-grade nanoemulsions: preparation, stability and application in encapsulation of bioactives. **Molecules**. doi.org/10.3390/molecules24234242

Marchese A, Orhan IE, Daglia M, et al. (2016) Antibacterial and antifungal activities of thymol: a literature review. **Food Chemistry**:402–414. doi.org/10.1016/j.foodchem.2016.04.111

Meenu M, Padhan B, Patel M, et al. (2023) Antibacterial activity of essential oils from different plant parts against *Salmonella* and *Listeria*. **Food Chemistry** 404. doi.org/10.1016/j.foodchem.2022.134723

Milbradt EL, Andreatti Filho RL (2020) Ácidos orgânicos na avicultura. In: Andreatti Filho RL et al., *Doenças das Aves*. Campinas: FACTA, 3ª ed., p. 115–136.

Moharreri M, Vakili R, Oskoueian E, et al. (2022) Effects of microencapsulated essential oils on broilers challenged with *Salmonella* Enteritidis. **J. Saudi Soc. Agric. Sci.** 21:349–357. doi.org/10.1016/j.jssas.2021.10.012

Murate LS, Paião FG, Almeida AM, et al. (2015) Efficacy of prebiotics, probiotics and symbiotics in hens and broilers challenged with *Salmonella* Enteritidis. **Jpn. Poult. Sci. Assoc.** 52:52–56. doi.org/10.2141/jpsa.0130211

Nuñez KVM, Abreu ACS, Almeida JM, et al. (2024) Antimicrobial activity of selected essential oils against *Staphylococcus aureus* from bovine mastitis. **Dairy** 5(1):54–65. doi.org/10.3390/dairy5010005

Oliveira Filho JGO, Duarte LGR, Silva YBB, et al. (2023) Papaya storage improvement using carnauba wax nanoemulsion with clove and spearmint essential oils. **Coatings**. doi.org/10.3390/coatings13050847

Oliveira GH, Berchieri Jr A, Barrow PA (2000) Prevention of *Salmonella* infection using adult bird flora and/or organic acids. **Braz. J. Microbiol.** 31:116–120. doi.org/10.1590/S1517-83822000000200011

Penteado AL, Eschionato RA, Souza DRC, et al. (2021) *In vitro* antimicrobial activity of essential oils against *Salmonella Typhimurium* and *Staphylococcus aureus*. **Revista Higiene Alimentar** 35(293). doi.org/10.37585/HA2021.02avaliacao

Rodrigues HLS, Benevides VP, Kolososki IMM, et al. (2025) Effective control of *Salmonella* Enteritidis in poultry by dietary microencapsulated essential oils. **Antibiotics** 14(6). doi.org/10.3390/antibiotics14060552

Saraiva MMS, Lim K, Monte DFM, et al. (2022) Antimicrobial resistance in the globalized food chain: A One Health perspective applied to poultry. **Braz. J. Microbiol.** 53:465–486. doi.org/10.1007/s42770-021-00635-8

Scharff RL (2020) Food attribution and economic cost estimates for meat- and poultry-related illnesses. **J. Food Protection** 83(6):959–967. doi.org/10.4315/JFP-19-548

Schrezenmeir J, Vrese M (2001) Probiotics, prebiotics and symbiotics – approaching a definition. **Am. J. Clin. Nutr.** 73:361–364. doi.org/10.1093/ajcn/73.2.361s

Severino R, Ferrari G, Vu KD, et al. (2015) Antimicrobial effects of chitosan coating with essential oil nanoemulsion and MAP against *E. coli* O157:H7 and *Salmonella* Typhimurium on green beans. **Food Control**:215–222. doi.org/10.1016/j.foodcont.2014.08.029

Silva FV, Guimarães AG, Silva ERS, et al. (2012) Anti-inflammatory and anti-ulcer activities of carvacrol. **J. Med. Food** 15(11). doi.org/10.1089/jmf.2012.0102

Sodagari HR, Wang P, Robertson I, et al. (2020) Non-typhoidal *Salmonella* at the human–food–animal interface in Australia. **Animals** 10(7). doi.org/10.3390/ani10071192

Somrani M, Huertas JP, Iguaz A, et al. (2023) Antimicrobial activity of cinnamon, garlic and onion essential oils against *Salmonella* Enteritidis biofilms. SSRN preprint. doi.org/10.2139/ssrn.4552975

Stingelin GM, Scherer RS, Machado AC, et al. (2023) Thymol, carvacrol and sorbic acid microcapsules to control *Salmonella* in broilers. **Front. Vet. Sci.** doi.org/10.3389/fvets.2022.1046395

Suresh G, Das RK, Kaur Brar S, et al. (2018) Alternatives to antibiotics in poultry feed: molecular perspectives. **Critical Reviews in Microbiology** 44(3):318–335. doi.org/10.1080/1040841X.2017.1373062

Thompson NT, Kitzenberg DA, Kao DJ (2023) Persister-mediated emergence of antimicrobial resistance due to antibiotic growth promoters. **AIMS Microbiology** 9(4):738–756. doi.org/10.3934/microbiol.2023038

WHO – World Health Organization (2023) *Antimicrobial resistance*. Disponível em: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Zancan FT, Berchieri Jr A, Fernandes SA, et al. (2000) *Salmonella* spp. investigation in transport boxes of day-old birds. **Braz. J. Microbiol.** 31:230–232. doi.org/10.1590/S1517-83822000000300016