



OPEN ACCESS

EDITED BY

Abdelaziz Ed-Dra,
Université Sultan Moulay Slimane,
Morocco

REVIEWED BY

Yanhong Liu,
United States Department of Agriculture
(USDA), United States
Keith Warriner,
University of Guelph, Canada

*CORRESPONDENCE

Kumar Venkitanarayanan
✉ kumar.venkitanarayanan@uconn.edu

RECEIVED 16 November 2025

REVISED 10 February 2026

ACCEPTED 10 February 2026

PUBLISHED 10 March 2026

CITATION

Viju LS, Joseph D, Kosuri VVPR,
Balasubramanian B, Allen J, Zhu C,
Shah T, Walunj A, Pellissery AJ, Mishra N,
Maas K and Venkitanarayanan K (2026)
Prophylactic efficacy of in-feed
supplementation of linalool for reducing
Salmonella Enteritidis colonization in
broiler chickens.
Front. Sustain. Food Syst. 10:1747665.
doi: 10.3389/fsufs.2026.1747665

COPYRIGHT

© 2026 Viju, Joseph, Kosuri,
Balasubramanian, Allen, Zhu, Shah,
Walunj, Pellissery, Mishra, Maas and
Venkitanarayanan. This is an
open-access article distributed under
the terms of the [Creative Commons
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,
distribution or reproduction in other
forums is permitted, provided the
original author(s) and the copyright
owner(s) are credited and that the
original publication in this journal is
cited, in accordance with accepted
academic practice. No use, distribution
or reproduction is permitted which does
not comply with these terms.

Prophylactic efficacy of in-feed supplementation of linalool for reducing *Salmonella* Enteritidis colonization in broiler chickens

Leya Susan Viju¹, Divya Joseph¹,
Veera Venkata Praveen Raja Kosuri¹,
Brindhalakshmi Balasubramanian¹, Jodie Allen¹, Chen Zhu¹,
Trushenkumar Shah¹, Atul Walunj¹, Abraham Joseph Pellissery²,
Neha Mishra³, Kendra Maas⁴ and Kumar Venkitanarayanan^{1*}

¹Department of Animal Science, University of Connecticut, Storrs, CT, United States, ²Department of Comparative, Diagnostic and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, United States, ³Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT, United States, ⁴Microbial Analysis, Resources, and Services (MARS) UConn CORE, University of Connecticut, Storrs, CT, United States

Salmonella Enteritidis (SE) is a significant foodborne pathogen, with chickens serving as its primary reservoir, underscoring the significance on-farm strategies for improving the microbiological safety of poultry products. This study evaluated the prophylactic efficacy of in-feed supplementation with linalool, a generally recognized as safe (GRAS) plant compound, in reducing SE colonization in broiler chickens. A total of 212 day-old broiler chicks were procured, with 192 randomly assigned to eight groups of 24 birds each, and 20 used to verify colonization efficiency post-challenge. The groups included a negative control (no SE, no linalool), compound controls (no SE, fed 1, 1.5%, or 1.8% linalool), a positive control (SE, no linalool), and treatment groups (SE, fed 1, 1.5%, or 1.8% linalool). On day 7, birds were inoculated with approximately 8.0 log₁₀ CFU of a four-strain SE mixture, and cecal colonization was determined 48 h later. Eight birds per group were euthanized on days 14, 24, and 34 to enumerate SE populations in the cecum, while liver and intestine samples were collected for histopathological analysis. Additionally microbiome analysis of cecal samples collected on days 24 and 34 was performed using 16S rRNA sequencing by Illumina Miseq. Moreover, the effect of linalool on select SE colonization and virulence genes was investigated by RT-qPCR. Results demonstrated that in-feed supplementation with 1.5 and 1.8% linalool significantly reduced SE counts by at least 2.5 to 3.0 log CFU/g cecum at 34 days ($p < 0.05$), without adversely affecting body weight, feed intake, or feed conversion ratio ($p > 0.05$). Cecal microbiome analysis showed no major disruptions in alpha or beta diversity due to linalool supplementation, indicating gut microbial stability. Furthermore, linalool at all tested concentrations did not induce histologic changes in liver or intestinal tissues, confirming its safety at the microscopic level. Histopathologic lesion scores in the liver and intestine were significantly lower in the SE + linalool 1.5% and SE + linalool 1.8% groups compared to the positive control ($p < 0.05$). Gene expression analysis revealed that linalool downregulated the expression of critical SE colonization genes ($p < 0.05$). These findings suggest that prophylactic in-feed supplementation with linalool effectively reduces SE colonization in broiler chickens without adversely affecting bird health and production parameters.

KEYWORDS

broiler chicken, linalool, natural antimicrobial, pre-harvest control, *Salmonella* Enteritidis, sustainable feed supplements

Introduction

The Food and Agriculture Organization (FAO) projects that poultry meat will account for nearly one-third of global meat production to meet the needs of a growing population expected to reach 10 billion by 2050 (FAO, 2009). The United States is currently the largest producer and second-largest exporter of poultry meat globally (Ufer et al., 2023). Given this increasing demand, ensuring the microbiological safety of poultry meat has become a priority for governments, industry stakeholders, and consumers alike.

Salmonellosis remains a major public health concern, causing an estimated 1.35 million infections, 26,500 hospitalizations, and 420 deaths annually in the U.S. (Boore et al., 2015; CDC, 2025; Lamichhane et al., 2024). Among the various serovars, *Salmonella enterica* serovar Enteritidis (SE) is the predominant strain associated with poultry and poultry products in the U. S. Chickens are the primary reservoir host for SE, with the cecum serving as the main site of colonization (Allen-Vercoe and Woodward, 1999; Foley et al., 2013; Pal et al., 2021). This colonization facilitates horizontal transmission, eggshell contamination through feces, and carcass contamination during slaughter (Keller et al., 1995; Lamichhane et al., 2024) as well as vertical transmission leading to egg contamination via the transovarian route (Gast et al., 2016; Shivaprasad et al., 1990). Poultry's critical role in SE transmission makes it one of the largest contributors to global salmonellosis cases (CDC, 2025; Kimura et al., 2004), as seen in outbreaks such as the multi-state occurrence in the U.S. in 2021 linked to chicken products (CDC, 2021). Given that SE can enter poultry production environments through various sources, including feed, water, bedding, equipment, personnel, and pests (Dougherty, 1976; Himathongkham et al., 1999; O'Bryan et al., 2022), pre-harvest control strategies are essential for enhancing the microbiological safety of poultry products. Reducing SE in the intestinal tract of chickens is a practical means to minimize contamination of poultry meat and eggs (Kuria, 2023; Marcus et al., 2007).

Many plant compounds, including terpenoids, phenolics, glycosides, alkaloids, flavonoids, and glucosinolates, have demonstrated antimicrobial properties through mechanisms such as membrane disruption and inhibition of pathogen colonization (Diaz-Sanchez et al., 2015). Linalool, an acyclic monoterpene alcohol found in the essential oils from plants such as lavender, basil, coriander, cinnamon, and rosewood (Aprotosoie et al., 2014; Singh and Mishra, 2024). Classified as generally recognized as safe (GRAS) by the U.S. FDA (FEMA, 1997), linalool offers both economic and functional advantages due to its abundance and proven *in vitro* efficacy against pathogens such as *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Zengin and Baysal, 2014; Soković et al., 2010). The widespread availability, low cost, palatability, and high water solubility of linalool further support its application in poultry diets. Previously, Beier et al. (2014) demonstrated linalool's antimicrobial properties and confirmed its safety when included in chicken feed at concentrations up to 2%, with no adverse effects on serum chemistry, organ health, or feed conversion.

Additionally, several mechanisms have been proposed to explain the antibacterial activity of linalool, including interactions with membrane phospholipids, membrane proteins, and specific intracellular targets (Guo et al., 2021; Maćzka et al., 2022). Upon contact with bacterial cells, linalool primarily targets the cell membrane, disrupting its function by reducing membrane potential and compromising membrane structure. This results in the leakage of macromolecules such as DNA, RNA, and proteins (An et al., 2021; Maćzka et al., 2022). Linalool has also demonstrated efficacy in dispersing and inactivating biofilms formed by *Listeria monocytogenes* (He et al., 2022). Linalool has also shown strong antimicrobial activity in post-harvest applications; when incorporated into pectin-based coatings for shell eggs, it produced nearly a 4-log reduction of *Salmonella* Heidelberg during refrigerated storage (Pellissery et al., 2022).

The use of sustainable, plant-derived feed supplements has gained increasing attention as poultry producers seek alternatives to conventional antibiotics that align with consumer expectations, regulatory restrictions, and antimicrobial-resistance mitigation strategies (Aminullah et al., 2025; Pitino et al., 2021). There is a growing interest in identifying sustainable, plant-derived feed supplements that can reduce reliance on conventional antibiotics while supporting food safety and production efficiency (Wang et al., 2024). Phytochemicals such as terpenoids, phenolics, and organic acids are particularly appealing because they are naturally occurring, biodegradable, widely available, and generally recognized as safe for use in poultry. These compounds have demonstrated the capacity to reduce enteric colonization by foodborne pathogens while maintaining growth performance and preserving gut microbial balance, making them suitable candidates for integration into sustainable poultry production systems (Aminullah et al., 2025). In this context, linalool represents a promising sustainable feed supplement because of its GRAS status, global availability, favorable safety profile, and proven antimicrobial activity.

Thus, linalool represents a promising phytochemical for controlling enteropathogens in poultry and aligns with the industry's need for natural alternatives to synthetic antimicrobials. The objective of this research was to determine the efficacy of linalool as a prophylactic in-feed supplement to reduce the colonization of SE in chickens.

Materials and methods

Plant-derived antimicrobial

Linalool((±)-3,7-dimethyl-1,6-octadien-3-ol,(±)-3,7-Dimethyl-3-hydroxy-1,6 octadiene, ≥97%, W263508 Sigma Aldrich, St. Louis, MO) was used.

Ethical statement

Day-old commercial Cornish Cross broiler chicks (Myers Poultry Farm, South Fork, PA) were housed in floor pens at the University of Connecticut's Avian Isolation Facility under age-appropriate temperature, ventilation, and bedding conditions.

Birds had ad libitum access to feed and water throughout the study, and all efforts were made to minimize discomfort through proper husbandry and handling practices. All experimental procedures, including pathogen challenge and euthanasia, were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Connecticut (Protocol A21-055).

At the end of the experiment, birds in each group were humanely euthanized using carbon dioxide (CO₂) inhalation, following the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (American Veterinary Medical Association, 2020) to ensure humane termination.

Bacterial strains and dosing

In this study, a cocktail mixture of four SE strains isolated from chickens was prepared. The strains included SE-12 (chicken liver, phage type 14b), SE-21 (chicken intestine, phage type 8), SE-28 (chicken ovary, phage type 13a), and SE-31 (chicken gut, phage type 13a) were obtained from the Department of Pathobiology and Veterinary Science, University of Connecticut. Each strain was pre-induced for resistance to 50 µg/mL nalidixic acid (NA; Sigma-Aldrich, St. Louis, MO) to facilitate selective enumeration (Johny et al., 2009). Lawn cultures of all four SE were grown on xylose lysine deoxycholate agar supplemented with nalidixic acid (XLD-NA, Difco) and incubated at 37 °C for 48 h. Following incubation, the bacterial cultures were transferred into 30 mL of sterile phosphate-buffered saline (PBS, pH 7.2), centrifuged at 3,000 × g for 15 min, and the resulting pellet was washed and resuspended in 25 mL of PBS. The suspension was mixed thoroughly using a vortex mixer to ensure homogeneity. Equal portions (8 log CFU/mL) from each of the four strains were combined to prepare a 4-strain mixture of the pathogen, and appropriate dilutions of this mixture were used to obtain the desired inoculum level.

Experimental design

We conducted a power analysis to determine the number of birds required for detecting a statistically significant difference in SE counts between the treatment and control, using the equation of Lenth (2009). We applied the Lenth (2009) sample-size framework with the following assumptions: $\alpha = 0.05$, $SD \approx 0.5\text{--}0.6$ log units, coefficient of variation $\approx 10\%$, expected biologically meaningful effect size ≥ 1.0 log CFU/g, and desired power = 0.80–0.90. Under these assumptions, a group size of approximately 7 birds per treatment per sampling day would be sufficient to detect a ≥ 1.0 log CFU/g difference with 80–90% power.

In the present study, 8 birds per treatment (1 additional bird to account for any mortality during the trial) were sampled on each sampling day (days 14, 24, and 34). The experimental design is depicted in Figure 1. Because the pen was considered the experimental unit, these 8 birds constituted subsamples used to generate one pen-level mean per treatment × day. Each treatment was replicated across three fully independent trials, yielding $n = 3$ biological replicates per treatment × day.

In each of the three trials, a total of 212-day-old broiler chicks were used. Of these, 192 birds were randomly allocated into eight experimental groups comprising of 24 birds each. The remaining 20 birds were utilized to evaluate the colonization efficiency of SE on day 2 post-challenge. The experimental groups included a negative control (no SE, no linalool), compound control (no SE, fed with 1, 1.5%, or 1.8% [vol/wt] linalool), a positive control (SE, no linalool), and treatment groups (SE, fed with 1, 1.5%, or 1.8% [vol/wt] linalool). A standard commercial broiler starter-grower feed (feed composition listed in Supplementary Table S1) was provided to all treatment groups, and linalool was supplemented daily by measuring the required amount of feed and mixing the corresponding volume of linalool (vol/wt) to achieve the designated inclusion levels. Linalool with a purity of $\geq 98\%$ (Sigma-Aldrich) was used in this study from day 1 to day 34 as an

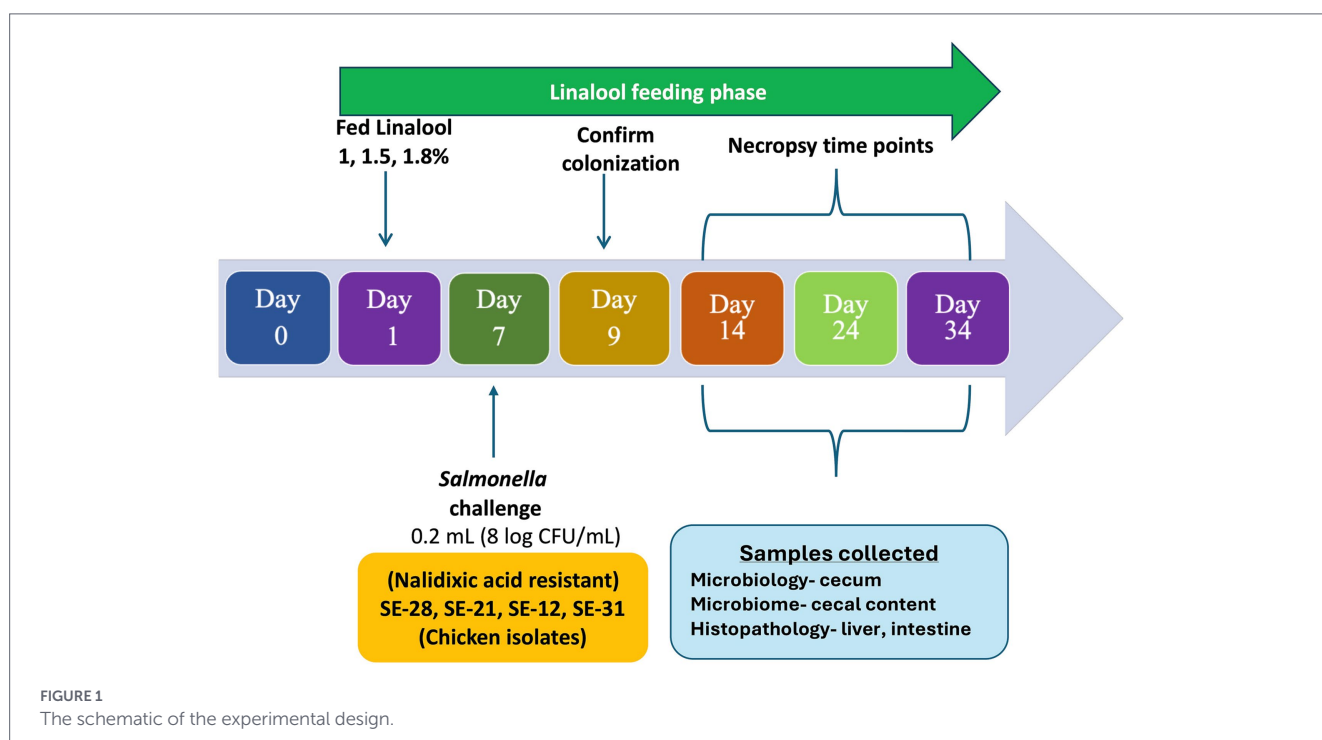


FIGURE 1
The schematic of the experimental design.

infeed supplement. On day 7, birds in the SE-challenged groups were orally inoculated with approximately 8.0_{10} log CFU of the four-strain SE mixture (200 μ L). Cecal colonization was assessed 48 h post-inoculation using 20 birds per experiment. Additionally, eight birds from each treatment group were euthanized on days 14, 24, and 34 post-challenge, and SE populations in the cecum were enumerated by plating on XLD-NA agar plates, following the method described by Johnny et al. (2009). Feed conversion ratio (FCR) was determined on days 14, 24, and 34 using cumulative feed intake (FI) and cumulative body-weight gain (BWG) (De Los Santos et al., 2008; Kurekci et al., 2014). Cumulative FI per bird was calculated from pen-level feed disappearance adjusted for the number of live birds. Cumulative BWG was computed as the change in mean body weight from day 0 and was measured individually per bird and averaged per pen.

Determination of SE in organs

Cecal samples from individual birds were aseptically collected into sterile Whirl-Pak[®] bags containing 10 mL of PBS. Each sample was weighed and homogenized using a tissue homogenizer (Tissue Master, Omni International, Marietta, GA), then subjected to serial 10-fold dilutions in sterile PBS. A 0.1 mL aliquot of the appropriate dilutions was surface-plated on duplicate on XLD-NA. The plates were incubated at 37 °C for 48 h, after which colony-forming units were enumerated. For samples in which no colonies were detected by direct plating, enrichment was performed by incubating the homogenized sample in 100 mL of selenite cysteine broth (Difco, Becton Dickinson) at 37 °C for 48 h, followed by streaking onto XLD-NA plates to confirm the presence of surviving SE.

Histopathological examination

Representative samples of liver and intestine from each group (8 birds each) were collected on days 14, 24, and 34 of necropsy and fixed in 10% neutral buffered formalin. Duplicate sections (5 mm thick) were cut from each sample and processed for histological examination using standard hematoxylin and eosin staining (Slaoui and Fiette, 2011). Tissues from birds that were not inoculated with SE and not treated with linalool were used as negative controls.

A board-certified veterinary pathologist performed blinded histopathological evaluations of all tissue sections. As the SE strains used in this study do not typically induce overt pathology in the liver or intestines of chickens, lesion scoring focused on subtle, yet relevant, microscopic changes. The evaluated criteria included: (1) bacterial coating of the enteric mucosa, (2) desquamation of enterocytes, (3) villous atrophy, (4) heterophil infiltration, and (5) hepatocellular degeneration (Shah et al., 2017). Each lesion was scored on a scale from 0 to 4, where 0 indicated no lesion, 1 minimal, 2 mild, 3 moderate, and 4 severe.

Microbiome analysis

For cecal microbiome analysis, cecal content (0.5 g) from 6 birds in each of the eight groups was collected in microcentrifuge tubes on days 24 and 34 of the bird trial and stored at -80 °C. Subsequent processing and analysis were conducted at the Microbial Analysis, Resources, and Services (MARS) at the University of Connecticut. Cecal samples were subjected to DNA extraction and 30 ng of the extracted DNA was used as the template for the amplification of the

partial bacterial 16S rRNA genes, targeting the V4-V5 region. The microbiome analysis was performed as a completely randomized design with treatments done in replicates of six, following published protocol. Mothur 1.36.1 was used to filter and cluster the sequences based on published protocol with slight modifications (Upadhyaya et al., 2024). Briefly, Operational taxonomic units (OTUs) were clustered at 97% sequence similarity. Downstream analysis of samples was conducted using R version 3.2. To calculate alpha diversity, inverse Simpson was used to measure the richness and evenness of the OTUs.

Determination of SICs of linalool

Subinhibitory concentrations (SICs) of linalool against SE were determined as described (Kollanoor-Johny et al., 2012). Sterile 24-well polystyrene plates (Costar; Corning, NY) containing Tryptic Soy Broth (TSB; 1 mL/well) were inoculated with ~ 6.0 log CFU of SE. Linalool (Sigma-Aldrich) was added at 1–10 μ L in 0.5 μ L increments. Plates were incubated at 37 °C for 24 h. Bacterial growth was assessed on duplicate Xylose Lysine Deoxycholate (XLD; Difco) agar plates. The SIC is defined as the highest concentration below the MIC that does not inhibit growth after 24 h compared with the untreated control (Sinel et al., 2017). All treatments were duplicated, and the experiment was repeated three times.

RNA isolation and RT-q-PCR analysis

Following SIC determination, SE cultures were grown with or without the SIC of linalool at 37 °C for 6 h (Upadhyaya et al., 2013). Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. RNA integrity and purity were confirmed by NanoDrop spectrophotometry ($A_{260}/A_{280} = 1.9$ – 2.1), and 1 μ g of RNA was used for cDNA synthesis with the SuperScript IV reverse transcriptase kit (Invitrogen, Carlsbad, CA). RT-qPCR was performed on an Applied Biosystems StepOnePlus Real-Time PCR System (StepOne Software v2.3) using SYBR Green Master Mix (Applied Biosystems). The selected SE colonization and virulence genes are given in Table 1.

Statistical analysis

For all *in vivo* measurements, the pen was considered the experimental unit. At each sampling time point (days 14, 24, and 34), eight birds were selected from the same treatment pen; bird-level observations were therefore treated as subsamples and were averaged to generate one pen-level value per treatment \times day. The experiment was repeated across three independent trials, yielding three biological replicates for each treatment \times day combination. Data from the three trials were pooled after confirming that no trial \times treatment interaction was present.

Data on bacterial counts, feed intake, feed conversion ratio, and body weight from three independent trials for the positive control and treatment groups were averaged and analyzed. Two-way analysis of variance (ANOVA) in GraphPad Prism 10 was used to detect differences among treatment means, and statistical significance was defined as $p < 0.05$. When significant main or interaction effects were detected, differences among treatment \times day means were evaluated using Tukey's multiple comparisons test. An overall histopathologic lesion score for each bird was calculated as the mean of the five individual lesion scores, and

TABLE 1 Genes selected for the SE transcriptional analysis.

Gene	Function	Primer	Sequence (5' → 3')
<i>invH</i>	Cell adherence and invasion (Li et al., 2009)	Forward	5'-CCCTTCCTCCGTGAGCAAA-3'
		Reverse	5'-TGGCCAGTTGCTCTTTCTGA-3'
<i>motA</i>	Salmonella motility (Li et al., 2009)	Forward	5'-AGCCGGAAATTTGCAGTGG-3'
		Reverse	5'-TCTTCCGGAACCTCCAGAT-3'
<i>hilA</i>	Regulation of Type III Secretion System (Zhou and Galán, 2001)	Forward	5'-CGCGGTTGCGTATTTTGTGA-3'
		Reverse	5'-CCGGGCTGGAAAGCATT-3'
<i>hilD</i>	Regulation of Type III Secretion System (Zhou and Galán, 2001)	Forward	5'-AGGCGCCGGCGTTGTGGA-3'
		Reverse	5'-TCAGACCTGGCTCTACCAGATG-3'
<i>sipA</i>	Effector protein promoting invasion and cytoskeletal rearrangement (Li et al., 2009)	Forward	5'-CAGGGAACGGTGTGGAGGTA-3'
		Reverse	5'-AGACGTTTTTGGGTGTGATACGT-3'
<i>sopB</i>	Uptake/invasion effector (Li et al., 2009)	Forward	5'-GCGTCAATTCATGGGCTAAC-3'
		Reverse	5'-GGCGGCGAACCTATAAACT-3'
<i>spvB</i>	ADP-ribosylating toxin affecting macrophage cytoskeleton (Lesnick et al., 2001; Otto et al., 2000)	Forward	5'-TGGGTGGGCAACAGCAA-3'
		Reverse	5'-GCAGGATGCCGTTACTGTCA-3'

group means were computed for comparative analysis. Statistical comparisons between groups were conducted using a two-sided Cochran-Armitage test for trend. To adjust for multiple comparisons, *p* values were corrected using the Benjamini and Hochberg method. A *p* < 0.05 was considered statistically significant.

Tukey's test was used to analyze the effect of both treatment and day on the alpha diversity. Beta-diversity was estimated as the difference in bacterial composition based on treatment and time by coupling Bray-Curtis Dissimilarity with non-metric multidimensional scaling (NMDS) for ordination from any resemblance matrix. A permutational multivariate analysis (PERMANOVA, adonis function, 999 permutations) was done to analyze the effect of various treatments on the bacterial community composition. Finally, the relative abundance of OTUs of major phyla, order, and genera were determined to assess the effect of treatment. To identify changes in groups of bacteria based on treatment, Tukey's test was used, and the significance was detected at *p* < 0.05. The RT-qPCR experiment was replicated three times. Differences among the means were considered significant at *p* < 0.05 and were detected using 2-way ANOVA in GraphPad Prism 10.

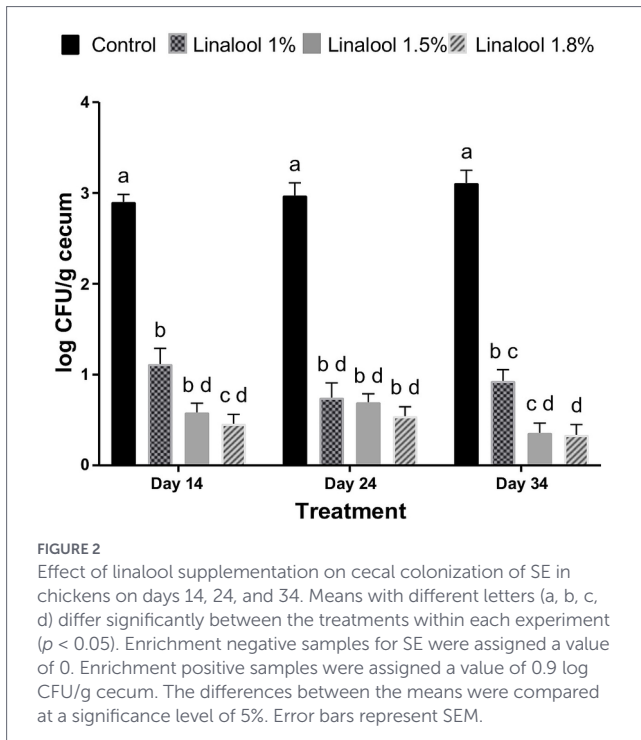
Results

Cecal colonization

S. Enteritidis was not detected in the unchallenged control groups (negative control and linalool control), confirming that these birds remained free of SE infection throughout the trial period. As shown in Figure 2, SE was recovered at approximately 3.0 log CFU/g cecum from positive control birds on days 14, 24, and 34. In contrast, linalool-treated birds exhibited significantly reduced SE counts in cecal samples on days 24 and 34 across all tested concentrations (1, 1.5, and 1.8%) (*p* < 0.05). On day 34, approximately 1 log CFU/g cecum of SE was recovered from the cecum of birds treated with 1% linalool, whereas the cecum from birds fed with 1.5 and 1.8% linalool did not yield any countable population of SE. However, these samples were positive for SE by enrichment.

Bird production parameters

No adverse effect noticed in body weight gain, feed intake, or feed conversion ratio (FCR) were observed between treated and



control birds (Table 2). Feed conversion ratio (FCR) was determined on days 14, 24, and 34 using cumulative feed intake (FI) and cumulative body-weight gain (BWG). Cumulative FI per bird was calculated from pen-level feed disappearance adjusted for the number of live birds. Cumulative BWG was computed as the change in mean body weight from day 0.

Cecal microbiome composition

Linalool supplementation did not significantly affect the alpha or beta diversity of the cecal microbiome ($p > 0.05$). The cecal microbial community was dominated by Firmicutes, *Lactobacillaceae*, *Lachnospiraceae*, and *Bacteroides* (Figure 3). Alpha diversity analysis using the Shannon index showed no significant differences between linalool-supplemented and control groups (Figure 4). β -diversity analysis using Bray–Curtis dissimilarity depicted clustering by sampling time points (days 24 and 34) as expected, but no significant separation between linalool-treated and control samples was observed (Figure 5).

Histopathological analysis

Histopathological examination of liver and intestinal tissues from the negative control and linalool-only groups (1, 1.5, and 1.8%) revealed normal tissue architecture, indicating that linalool at these concentrations did not induce microscopic alterations in these tissues. In contrast, the positive control group (SE-challenged, no linalool) exhibited mild to moderate lesions in the liver and intestine, consistent with SE infection (Figure 6). However, linalool supplementation at 1.5 and 1.8% significantly reduced histopathologic lesion scores compared to the positive control group ($p < 0.05$), as shown in Figure 7. Subtle microscopic changes in

TABLE 2 Body weight gain (BWG), cumulative feed intake (FI), and feed conversion ratio (FCR) of chickens fed control and linalool diets under SE challenge on days 14, 24, and 34.

Day	Treatment	BWG (kg)	FI (kg)	FCR
14	Control	0.50	0.92	1.84
	Linalool 1.5%	0.51	0.90	1.77
	Linalool 1.8%	0.45	0.85	1.88
24	Control	1.51	2.45	1.62
	Linalool 1.5%	1.41	2.35	1.67
	Linalool 1.8%	1.36	2.21	1.63
34	Control	2.15	3.50	1.63
	Linalool 1.5%	2.10	3.45	1.64
	Linalool 1.8%	2.10	3.43	1.63

Feed conversion ratio (FCR) was determined at days 14, 24, 34, and 42 using cumulative feed intake (FI) and cumulative body-weight gain (BWG). FI per bird was calculated from pen-level feed disappearance adjusted for the number of live birds. BWG was computed as the change in mean body weight from day 0. FCR was expressed as: $FCR = \frac{FI(\text{kg per bird})}{BWG(\text{kg per bird})}$.

SE + linalool groups included reduced bacterial coating of the enteric mucosa and decreased heterophilic infiltration in the lamina propria of the small intestine. No histopathologic lesions were observed in any group not challenged with SE, further confirming the absence of infection.

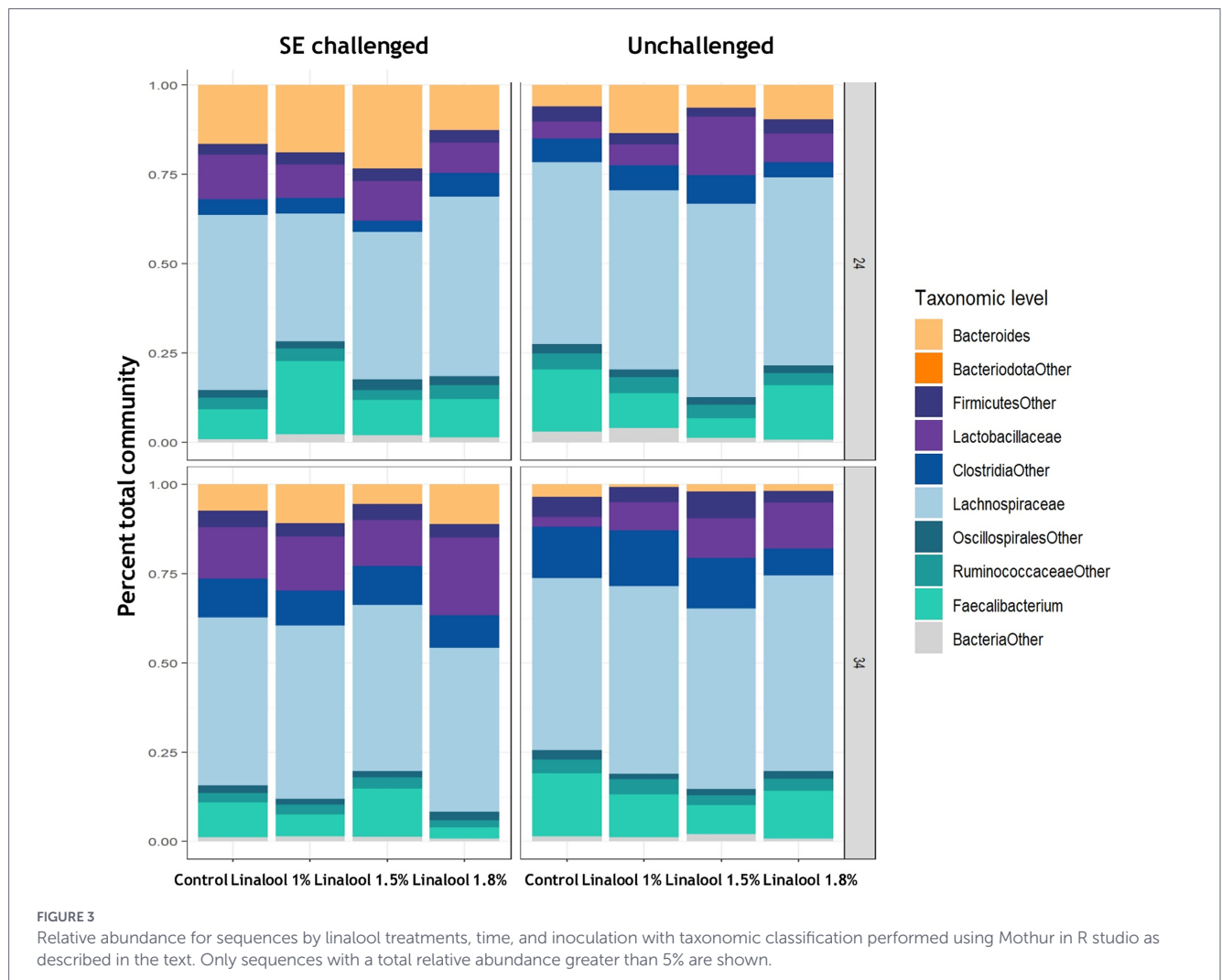
Effect of linalool on transcription of colonization and virulence genes in SE

The SIC of linalool against SE was determined to be 1.62 mM (0.025%). Quantitative real-time PCR (RT-qPCR) analysis revealed that SIC of linalool significantly downregulated the transcription of key virulence and colonization-associated genes in both SE isolates ($p < 0.05$) (Figures 8A,B). The targeted genes included those involved in bacterial adhesion, invasion, motility, and host cell dissemination and immune evasion.

In SE, the transcription of selected genes was consistently downregulated across both strains tested, with the magnitude of fold-change reduction being relatively uniform.

Discussion

The results of this study demonstrate that linalool supplementation at all three tested concentrations consistently reduced SE populations in the cecum. Overall, linalool exhibited a concentration-dependent effect, with the 1.5 and 1.8% treatments reducing SE levels to below 1 log CFU/g cecum at all three sampling points. Although the numerical difference between 1 and 1.5% linalool appears small, such narrow incremental changes in dietary phytochemical concentrations have previously been shown to produce nonlinear inhibitory effects on enteric pathogens due to threshold-based interactions. Previously, a study by Upadhyaya et al. (2015) reported that the dietary supplementation of trans-cinnamaldehyde (TC) in broiler chickens at 1 and 1.5% significantly reduced



the cecal counts of SE ($p < 0.001$) compared to those in control birds that did not receive TC. Similarly, the efficacies of TC and eugenol (EG) in reducing SE colonization in broiler chickens have been well documented in another study, with TC at 0.5 or 0.75% and EG at 0.75 or 1% producing consistent reductions in cecal *Salmonella* levels (Kollanoor-Johny et al., 2012). Similar dose-response reductions in pathogen counts have been documented for other plant-derived antimicrobials in poultry, where relatively small increases in dietary thymol, oregano essential oils, cinnamaldehyde, or caprylic acid shift outcomes from minimal to marked reductions in enteric pathogen loads (Ibrahim et al., 2021; Hu et al., 2023).

There were no adverse effects observed in feed consumption, body weight and FCR between linalool-treated and control birds. Additionally, histopathological examination of liver and intestinal tissues from the negative control and linalool-only groups (1, 1.5, and 1.8%) revealed normal tissue architecture, indicating that linalool at these concentrations did not induce microscopic alterations in these tissues. This suggests that dietary linalool did not elicit detectable local or systemic toxicity. This finding supports the *in vivo* safety of linalool as a feed additive and aligns with previous reports describing minimal histopathological changes in broilers (Beier et al., 2014). Additionally, birds supplemented with linalool at 1.5 and 1.8% showed significantly lower histopathologic lesion

scores in both liver and intestinal tissues compared to the positive control group ($p < 0.05$). These findings suggest that linalool, even at higher inclusion rates, effectively reduced SE colonization in birds without negatively impacting broiler performance or tissue integrity.

Intestinal health in poultry is essential for efficient digestion, optimal nutrient absorption, a robust immune response, and overall growth and performance (Aruwa et al., 2021; Ducatelle et al., 2023). Therefore, any antimicrobial feed additive aimed at reducing SE colonization should not negatively impact the gut microbiome. In this study, microbiome analysis of cecal contents collected on days 24 and 34 indicated no adverse effects on the gut microbiota, as evidenced by alpha and beta diversity metrics. Notably, the relative abundance data revealed an increase in the *Lactobacillaceae* family in linalool-supplemented birds at both time points. This increase may be beneficial, as *Lactobacillaceae* have been associated with improved nutrient absorption, enhanced gut barrier function, and overall intestinal health (Ciorba, 2012; Yin et al., 2023).

The exact mechanism behind the selective inhibitory effects of linalool on *Salmonella* with no apparent effects on the normal gut flora is not known. Several studies have shown that many plant-derived antimicrobials exhibit selective activity against enteric pathogens while exerting minimal effects on beneficial commensals. In the study done by Upadhyaya et al. (2024), the effect of

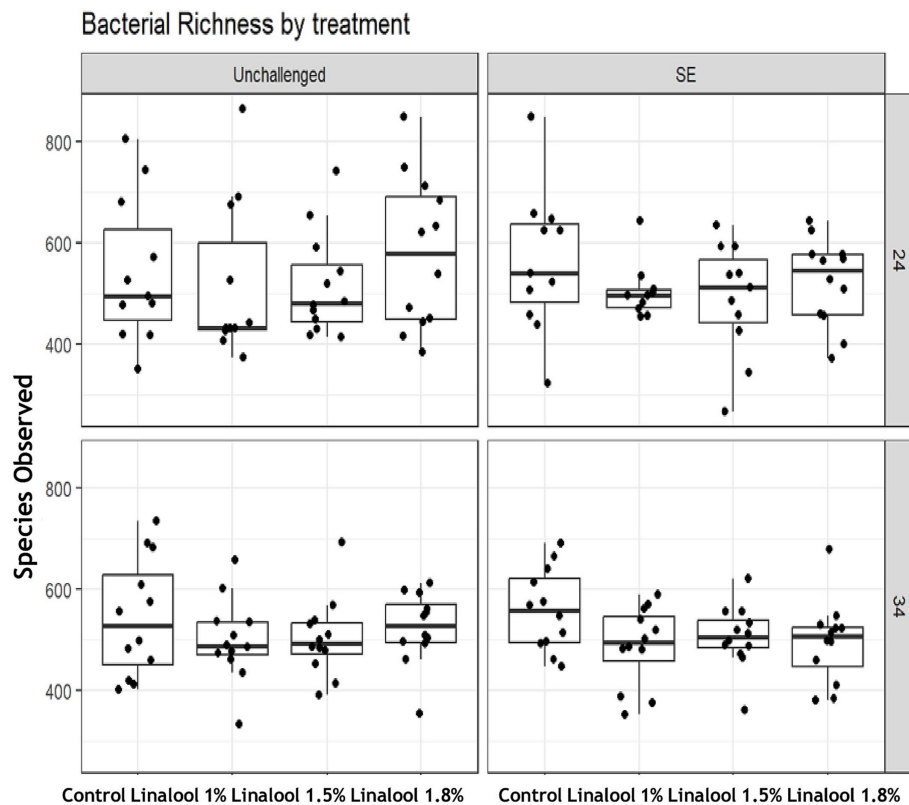


FIGURE 4 Effect of linalool on the alpha diversity of cecal microbiota communities of chickens on days 24 and 34. The box plots show Shannon indices of treatments on the challenged and unchallenged with SE groups as described in the text.

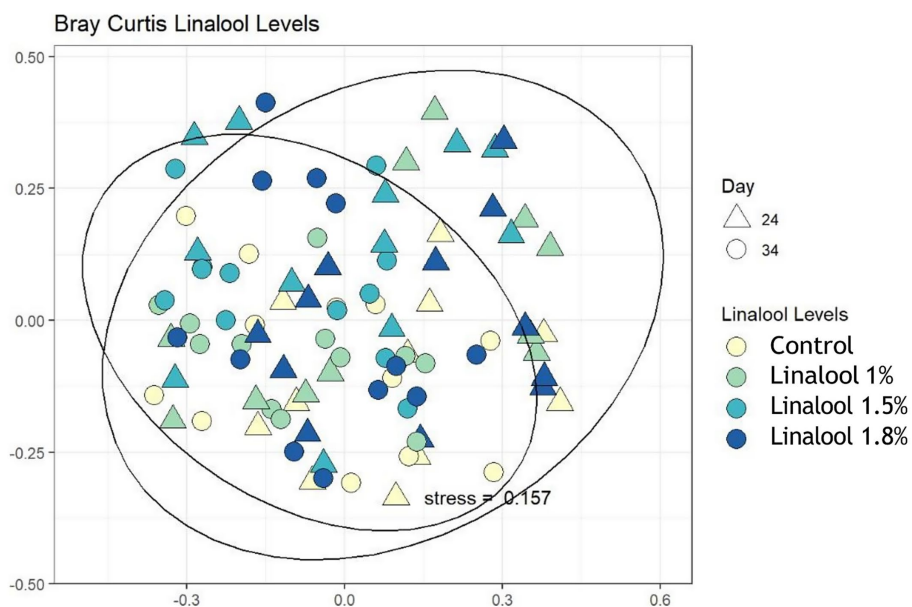


FIGURE 5 Bray-Curtis plot showing the effect of linalool on the beta diversity of cecal microbiota communities of chickens at days 24 and 34.

in-feed supplementation of two phytochemicals, Trans-cinnamaldehyde and Caprylic acid, did not affect the cecal population of the major bacterial phylotypes. It has also been reported that dietary organic acids and coated essential oils markedly reduced *E. coli*, *Salmonella*, and *Clostridium perfringens* in broilers

while producing no major shifts in overall microbial community structure, and also while *Lactobacillus* growth was positively improved (Islam et al., 2024). Also, TC at 0.5–0.75% and EG at 0.75–1% consistently lowered cecal *Salmonella* counts, but did not markedly disrupt the overall endogenous bacterial populations

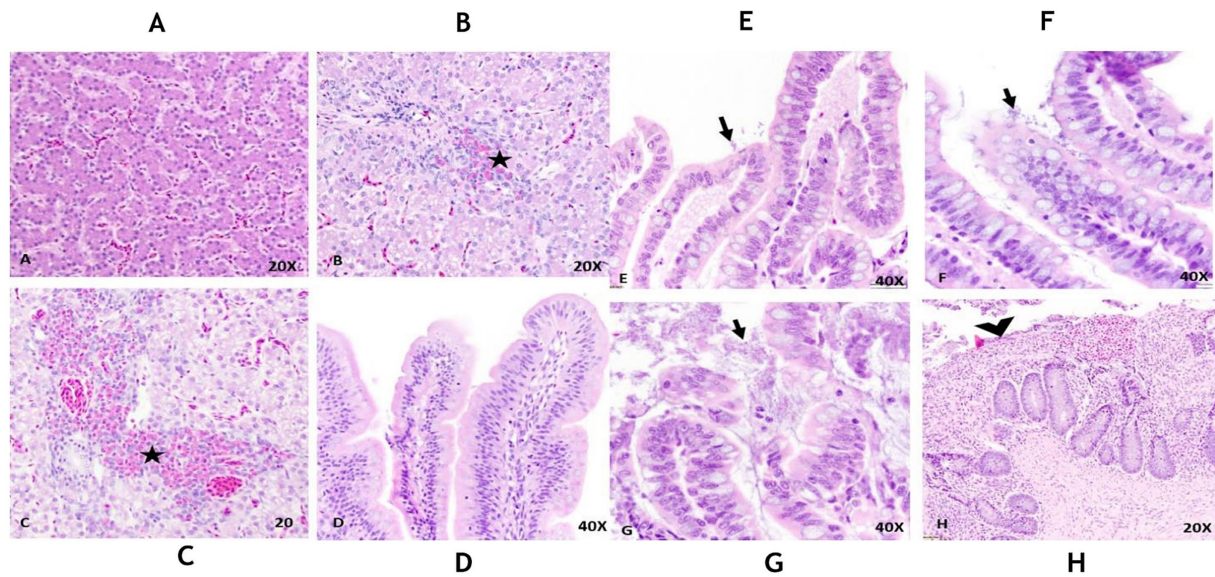


FIGURE 6

Depiction of histology in liver and intestinal tissues from experimental groups of chickens. (A) Histology of liver from negative control group (no SE, no linalool), no lesion noted, hematoxylin and eosin (H&E) staining, 20X magnification. Histology of liver from linalool only (no SE) 1, 1.5, and 1.8% groups showed similar normal histology; (B) Histology of liver from SE + linalool 1% (linalool treatment) group showing milder infiltration of hepatic parenchyma with heterophils (asterisk), hepatocellular degeneration as compared to (C) PC: positive control (SE, no linalool) group, hematoxylin and eosin (H&E) staining, 20X magnification. (D) Histology of small intestine from negative control group (no SE, no linalool), no lesion noted, hematoxylin and eosin (H&E) staining, 40X magnification. (E) SE + linalool 1.8%, (F) SE + linalool 1.5%, (G) SE + linalool 1% groups show reduced bacterial coating (arrow) of enteric mucosa (rare, small numbers and moderate numbers, respectively), (hematoxylin and eosin (H&E) staining, 40X magnification) as compared to the positive control group which is shown in (H) PC: positive control (SE, no linalool) group small intestine shows evidence of desquamation of enterocytes (arrow head), bacterial coating of enteric mucosa, inflammation, and villous atrophy (hematoxylin and eosin (H&E) staining, 20X magnification).

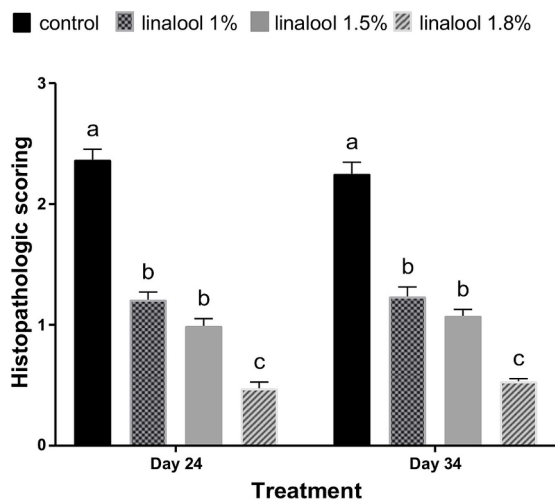


FIGURE 7

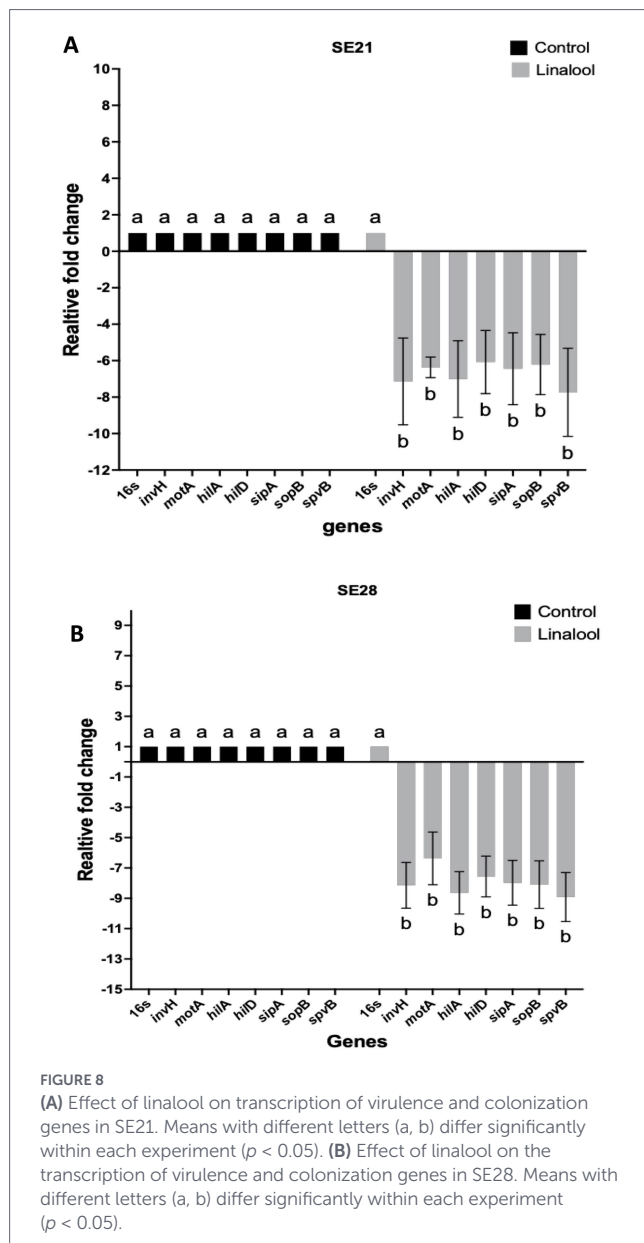
Cumulative histopathologic lesion scores of livers and intestines on days 24 and 34. Means with different letters (a-c) differ significantly between the treatments within each experiment ($p < 0.05$).

within the cecum (Kollanoor-Johny et al., 2012). All these findings point toward that certain antimicrobial feed additives can suppress specific pathogens without broadly altering gut microbiota composition.

Sub-inhibitory concentrations (SICs) of antimicrobials, including antibiotics, are known to modulate bacterial physicochemical functions by altering gene expression (Laureti et al., 2013).

Therefore, we examined the effects of SIC of linalool on the transcription of virulence and colonization genes in SE *in vitro* using RT-qPCR. The RT-qPCR results revealed that linalool significantly downregulated ($p < 0.05$) key virulence and colonization genes in both SE isolates. The downregulated genes included motility gene *motA*; invasion genes *hilA*, *hilD*, and *invF*; the pathogenicity island 1 effector protein gene *sipA*; the cell invasion gene *sopB*; and the toxin production gene *spvB* (Li et al., 2009). Because these genes are involved in flagellar function, epithelial attachment, invasion, and early intracellular survival, their suppression suggests that linalool may attenuate multiple steps of SE pathogenesis. This transcriptional downregulation of virulence pathways likely contributed to the reduced SE load in treated birds. These findings support the hypothesis that linalool exerts its inhibitory effects on SE at least in part, by modulating gene expression involved in bacterial colonization and virulence, thereby potentially reducing the ability of SE to establish infection in the host gastrointestinal tract.

Although this study demonstrates the potential efficacy of dietary linalool in reducing SE carriage in broiler chickens, a few limitations should be considered. One important limitation is the need to evaluate the economic feasibility and scalability of including linalool at the tested dietary levels within commercial production systems. Additionally, while no negative impacts on growth performance were observed, the longer-term consequences of linalool supplementation on immune function and meat quality remain unknown. The trials were also carried out in controlled research settings using a single broiler genotype; therefore, the results may not fully reflect outcomes in commercial environments, where diverse management practices, environmental



stressors, and complex microbiota could influence treatment responses. Despite these constraints, this study represents the first demonstration of linalool's potential as a feed-based strategy to reduce SE colonization in broiler chickens. Future research will aim to confirm its effectiveness in larger bird populations under field conditions and to assess its implications for bird health, performance, and carcass characteristics.

In conclusion, prophylactic supplementation of linalool, particularly at 1.5 and 1.8% concentrations proved effective in reducing cecal colonization of SE in broiler chickens, without compromising bird health and production performance. These findings support the potential use of linalool as a natural antimicrobial feed additive, especially when integrated with on-farm hygienic practices, to enhance the microbiological safety of poultry products. However, follow up large-scale studies under commercial field conditions are warranted to validate these results and assess long-term efficacy and feasibility.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: <https://www.ncbi.nlm.nih.gov/SAMN51424741>.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee (IACUC) at the University of Connecticut. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

LV: Data curation, Writing – review & editing, Investigation, Formal analysis, Writing – original draft, Visualization. DJ: Writing – review & editing, Investigation. VK: Writing – review & editing. BB: Writing – review & editing. JA: Writing – review & editing. CZ: Writing – review & editing. TS: Writing – review & editing. AW: Writing – review & editing. AP: Writing – review & editing. NM: Formal analysis, Writing – review & editing, Visualization. KM: Visualization, Writing – review & editing. KV: Resources, Supervision, Funding acquisition, Writing – review & editing, Project administration, Conceptualization, Validation, Writing – original draft, Methodology.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This research was supported by a USDA-Sustainable Agricultural Systems grant (2020-69012-31823) awarded to KV.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that Generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy,

including review by the authors wherever possible. If you identify any issues, please contact us.

that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product

Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2026.1747665/full#supplementary-material>

References

- Allen-Vercoe, E., and Woodward, M. J. (1999). Colonisation of the chicken caecum by afimbriate and flagellate derivatives of *Salmonella enterica* serotype Enteritidis. *Vet. Microbiol.* 69, 265–275. doi: 10.1016/S0378-1135(99)00114-5
- American Veterinary Medical Association (2020). *AVMA guidelines for the euthanasia of animals: 2020 edition*. Schaumburg, IL: American Veterinary Medical Association.
- Aminullah, N., Mostamand, A., Zahir, A., Mahaq, O., and Azizi, M. N. (2025). Phyto-genic feed additives as alternatives to antibiotics in poultry production: a review. *Veterinary World* 18, 141–154. doi: 10.14202/vetworld.2025.141-154
- An, Q., Ren, J.-N., Li, X., Fan, G., Qu, S.-S., Song, Y., et al. (2021). Recent updates on bioactive properties of linalool. *Food Funct.* 12, 10370–10389. doi: 10.1039/D1FO02120F
- Aprotosoia, A. C., Hăncianu, M., Costache, I.-I., and Miron, A. (2014). Linalool: a review on a key odorant molecule with valuable biological properties. *Flavour Fragr. J.* 29, 193–219. doi: 10.1002/ffj.3197
- Aruwa, C. E., Pillay, C., Nyaga, M. M., and Sabiu, S. (2021). Poultry gut health—microbiome functions, environmental impacts, microbiome engineering and advancements in characterization technologies. *J. Anim. Sci. Biotech.* 12:119. doi: 10.1186/s40104-021-00640-9
- Beier, R. C., Byrd, J. A., Kubena, L. F., Hume, M. E., McReynolds, J. L., Anderson, R. C., et al. (2014). Evaluation of linalool, a natural antimicrobial and insecticidal essential oil from basil: effects on poultry. *Poult. Sci.* 93, 267–272. doi: 10.3382/ps.2013-03254
- Boore, A. L., Hoekstra, R. M., Iwamoto, M., Fields, P. I., Bishop, R. D., and Swerdlow, D. L. (2015). *Salmonella enterica* infections in the United States and assessment of coefficients of variation. *PLoS One* 10:e0145416. doi: 10.1371/journal.pone.0145416
- CDC. (2021). 2021 Salmonella outbreak linked to raw frozen breaded stuffed chicken products. Available online at: https://archive.cdc.gov/www_cdc_gov/salmonella/enteritidis-06-21/index.html (Accessed September 2, 2025).
- CDC. (2025). Reports of selected Salmonella outbreak investigations. Available online at: <https://www.cdc.gov/salmonella/outbreaks/index.html> (Accessed 2 September 2025).
- Ciorba, M. A. (2012). A gastroenterologist's guide to probiotics. *Clin. Gastroenterol. Hepatol.* 10, 960–968. doi: 10.1016/j.cgh.2012.03.024
- de Los Santos, F. S., Donoghue, A. M., Venkitanarayanan, K., Dirain, M. L., Reyes-Herrera, I., Blore, P. J., et al. (2008). Caprylic acid supplemented in feed reduces enteric *Campylobacter jejuni* colonization in ten-day-old broiler chickens. *Poult. Sci.* 87, 800–804. doi: 10.3382/ps.2007-00280
- Diaz-Sanchez, S., D'Souza, D., Biswas, D., and Hanning, I. (2015). Botanical alternatives to antibiotics for use in organic poultry production. *Poult. Sci.* 94, 1419–1430. doi: 10.3382/ps/pev014
- Dougherty, T. J. (1976). Study of *Salmonella* contamination in broiler flocks. *Poult. Sci.* 55, 1811–1815. doi: 10.3382/ps.0551811
- Ducattelle, R., Goossens, E., Eeckhaut, V., and Van Immerseel, F. (2023). Poultry gut health and beyond. *Animal Nutrition* 13, 240–248. doi: 10.1016/j.aninu.2023.03.005
- FAO. (2009). How to feed the world in 2050. Available online at: https://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050_Global_Agriculture.pdf (Accessed September 2, 2025).
- FEMA (1997). *FEMA database: linalool (FEMA No. 2635)*. Washington, DC: Flavor and Extract Manufacturers' Association.
- Foley, S. L., Johnson, T. J., Ricke, S. C., Nayak, R., and Danzeisen, J. (2013). *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiol. Mol. Biol. Rev.* 77, 582–607. doi: 10.1128/MMBR.00015-13
- Gast, R. K., Guraya, R., Jones, D. R., Anderson, K. E., and Karcher, D. M. (2016). Colonization of internal organs by *Salmonella enteritidis* in enriched cages. *Poult. Sci.* 95, 1363–1369. doi: 10.3382/ps/pew037
- Guo, F., Chen, Q., Liang, Q., Zhang, M., Chen, W., Chen, H., et al. (2021). Antimicrobial activity and proposed mechanism of linalool against *Pseudomonas fluorescens*. *Front. Microbiol.* 12:562094. doi: 10.3389/fmicb.2021.562094
- He, R., Chen, W., Chen, H., Zhong, Q., Zhang, H., Zhang, M., et al. (2022). Antibacterial mechanism of linalool against *Listeria monocytogenes*: a metabolomic study. *Food Control* 132:108533. doi: 10.1016/j.foodcont.2021.108533
- Himathongkham, S., Nuanualsuwan, S., and Riemann, H. (1999). Survival of *S. Enteritidis* and *S. typhimurium* in chicken manure. *FEMS Microbiol. Lett.* 172, 159–163. doi: 10.1111/j.1574-6968.1999.tb13464.x
- Hu, Z., Liu, L., Guo, F., Huang, J., Qiao, J., Bi, R., 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. Biotech.* 14:95. doi: 10.1186/s40104-023-00889-2
- Ibrahim, D., Abdelfattah-Hassan, A., Badawi, M., El-Mandrawy, S. A., El-Sayed, S. A. A., Dawood, M. A. O., et al. (2021). Thymol nanoemulsion promoted broiler chicken's growth, gastrointestinal barrier and bacterial community and conferred protection against *Salmonella Typhimurium*. *Sci. Rep.* 11:7742. doi: 10.1038/s41598-021-86990-w
- Islam, Z., Sultan, A., Khan, S., Khan, K., Jan, A. U., Aziz, T., 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, 152–163. doi: 10.1080/1828051x.2023.2297562
- Johny, A. K., Baskaran, S. A., Charles, A. S., Amalaradjou, M. A. R., Darre, M. J., Khan, M. I., et al. (2009). Caprylic acid supplementation reduces *Salmonella enteritidis* colonization. *J. Food Prot.* 72, 722–727. doi: 10.4315/0362-028X-72.4.722
- Keller, L. H., Benson, C. E., Krotec, K., and Eckroade, R. J. (1995). *Salmonella enteritidis* colonization of the reproductive tract and eggs. *Infect. Immun.* 63, 2443–2449. doi: 10.1128/iai.63.7.2443-2449.1995
- Kimura, A. C., Reddy, V., Marcus, R., Cieslak, P. R., Mohle-Boetani, J. C., Kassenborg, H. D., et al. (2004). Chicken consumption as a risk factor for sporadic *S. enteritidis*. *Clin. Infect. Dis.* 38, S244–S252. doi: 10.1086/381576
- Kollanoor-Johny, A., Mattson, T., Baskaran, S. A., Amalaradjou, M. A. R., Babapoor, S., March, B., et al. (2012). Reduction of *Salmonella enterica* serovar Enteritidis colonization in 20-day-old broiler chickens by the plant-derived compounds trans-cinnamaldehyde and eugenol. *Appl. Environ. Microbiol.* 78, 2981–2987. doi: 10.1128/AEM.07643-11
- Kurekci, C., Al Jassim, R., Hassan, E., Bishop-Hurley, S. L., Padmanabha, J., and McSweeney, C. S. (2014). Effects of feeding plant-derived agents on the colonization of *Campylobacter jejuni* in broiler chickens. *Poult. Sci.* 93, 2337–2346. doi: 10.3382/ps.2014-03950
- Kuria, J. K. N. (2023). “Salmonellosis in food and companion animals and its public health importance”, in *Salmonella – Perspectives for Low-Cost Prevention, Control and Treatment*. IntechOpen. doi: 10.5772/intechopen.109324
- Lamichhane, B., Mawad, A. M. M., Saleh, M., Kelley, W. G., Harrington, P. J., Lovestad, C. W., et al. (2024). Salmonellosis: epidemiology, pathogenesis, and mitigation of AMR. *Antibiotics* 13:76. doi: 10.3390/antibiotics13010076
- Lauretli, L., Matic, I., and Gutierrez, A. (2013). Subinhibitory antibiotics induce genome instability. *Antibiotics* 2, 100–114. doi: 10.3390/antibiotics2010100
- Lenth, R. V. (2009). Java applets for power and sample size [computer software]. Available online at: <http://www.stat.uiowa.edu/~rlenth/Power>
- Lesnick, M. L., Reiner, N. E., Fierer, J., and Guiney, D. G. (2001). The *Salmonella* spvB virulence gene encodes an enzyme that ADP-ribosylates actin and destabilizes the cytoskeleton of eukaryotic cells. *Mol. Microbiol.* 39, 1464–1470. doi: 10.1046/j.1365-2958.2001.02340.x
- Li, S., Zhang, Z., Pace, L., Lillehoj, H., and Zhang, S. (2009). Functions exerted by the virulence-associated type-three secretion systems during *Salmonella enterica* serovar Enteritidis invasion into and survival within chicken oviduct epithelial cells and macrophages. *Avian Pathol.* 38, 97–106. doi: 10.1080/03079450902737782
- Mączka, W., Duda-Madej, A., Grabarczyk, M., and Wińska, K. (2022). Natural compounds—linalool. *Molecules* 27:6928. doi: 10.3390/molecules27206928
- Marcus, R., Varma, J. K., Medus, C., Boothe, E. J., Anderson, B. J., Crume, T., et al. (2007). Re-assessment of risk factors for sporadic *Salmonella* serotype Enteritidis infections: a

- case-control study in five FoodNet sites, 2002–2003. *Epidemiol. Infect.* 135, 84–92. doi: 10.1017/S0950268806006558
- O'Bryan, C. A., Ricke, S. C., and Marcy, J. A. (2022). Public health impact of *Salmonella* spp. on raw poultry: current concepts and future prospects in the United States. *Food Control* 132:108539. doi: 10.1016/j.foodcont.2021.108539
- Otto, H., Tezcan-Merdol, D., Girisch, R., Haag, F., Rhen, M., and Koch-Nolte, F. (2000). The *spvB* gene product is a mono(ADP-ribosyl)transferase. *Mol. Microbiol.* 37, 1106–1115. doi: 10.1046/j.1365-2958.2000.02064.x
- Pal, A., Riggs, M. R., Urrutia, A., Osborne, R. C., Jackson, A. P., Bailey, M. A., et al. (2021). Aerosolized *S. enteritidis* colonization in broilers. *Poult. Sci.* 100:101504. doi: 10.1016/j.psj.2021.101504
- Pellissery, A. J., Vinayamohan, P. G., Xue, J., Wang, X., Viju, L. S., Joseph, D., et al. (2022). Efficacy of pectin-based caproic acid, caprylic acid, linalool, and cuminaldehyde coatings in reducing *Salmonella* Heidelberg on chicken eggs. *Front. Sustain. Food Syst.* 6:874219. doi: 10.3389/fsufs.2022.874219
- Pitino, R., De Marchi, M., Manuelian, C. L., Johnson, M., Simoni, M., Righi, F., et al. (2021). Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins on yield, quality, and oxidative status of poultry products: a review of the literature of the last 20 years. *Antioxidants* 10:757. doi: 10.3390/antiox10050757
- Shah, D. H., Elder, J. R., Chiok, K. L., and Paul, N. C. (2017). "Genetic basis of *S. enteritidis* pathogenesis in chickens" in *Producing safe eggs*. eds. S. C. Ricke and R. K. Gast (Academic Press), 187–208.
- Shivaprasad, H. L., Timoney, J. F., Morales, S., Lucio, B., and Baker, R. C. (1990). Pathogenesis of *S. enteritidis* in laying hens. *Avian Dis.* 34, 548–557. doi: 10.2307/1591243
- Sinel, C., Cacaci, M., Meignen, P., Guérin, F., Davies, B. W., Sanguinetti, M., et al. (2017). Subinhibitory concentrations of ciprofloxacin enhance antimicrobial resistance and pathogenicity of *Enterococcus faecium*. *Antimicrob. Agents Chemother.* 61, e02763–e02716. Available at: doi: 10.1128/AAC.02763-16
- Singh, S., and Mishra, A. (2024). Linalool therapeutic applications. *Drug Res.* 74, 255–268. doi: 10.1055/a-2321-9571
- Slaoui, M., and Fiette, L. (2011). "Histopathology procedures" in *Drug safety evaluation: methods and protocols*. ed. J.-C. Gautier (Humana Press), 69–82. doi: 10.1007/978-1-60761-849-2_4
- Soković, M., Glamočlija, J., Marin, P. D., Brkić, D., and Van Griensven, L. J. (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules* 15, 7532–7546. doi: 10.3390/molecules15117532
- Ufer, D. J., Padilla, S., and Link, N. (2023). *U.S. trade performance in meat & poultry*: ERR-312.
- Upadhyaya, I., Upadhyay, A., Chen, C. H., Yin, H. B., Nair, M. S., Maas, K., et al. (2024). Cinnamaldehyde and caprylic acid effects on cecal microbiome. *Arch. Anim. Poult. Sci.* 2:555592. doi: 10.19080/AAPS.2024.02.555592
- Upadhyaya, I., Upadhyay, A., Kollanoor-Johny, A., Darre, M. J., and Venkitanarayanan, K. (2013). Effect of plant-derived antimicrobials on *Salmonella Enteritidis* adhesion to and invasion of primary chicken oviduct epithelial cells in vitro and virulence gene expression. *Int. J. Mol. Sci.* 14, 10608–10625. doi: 10.3390/ijms140510608
- Upadhyaya, I., Upadhyay, A., Kollanoor-Johny, A., Mooyottu, S., Baskaran, S. A., Yin, H. B., et al. (2015). In-feed supplementation of trans-cinnamaldehyde reduces layer-chicken egg-borne transmission of *Salmonella enterica* serovar Enteritidis. *Appl. Environ. Microbiol.* 81, 2985–2994. doi: 10.1128/AEM.03809-14
- Wang, J., Deng, L., Chen, M., Che, Y., Li, L., Zhu, L., et al. (2024). Phytochemical feed additives as natural antibiotic alternatives in animal health and production: a review of the literature of the last decade. *Animal Nutrition* 17, 244–264. doi: 10.1016/j.aninu.2024.01.012
- Yin, Y., Liao, Y., Li, J., Pei, Z., Wang, L., Shi, Y., et al. (2023). 'Lactobacillus plantarum GX17 benefits growth performance and improves functions of intestinal barrier/intestinal flora among yellow-feathered broilers. *Front. Immunol.* 14:1195382. doi: 10.3389/fimmu.2023.1195382
- Zengin, H., and Baysal, A. H. (2014). Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules* 19, 17773–17798. doi: 10.3390/molecules191117773
- Zhou, D., and Galán, J. (2001). *Salmonella* entry into host cells: the work in concert of type III secreted effector proteins. *Microbes Infect.* 3, 1293–1298. doi: 10.1016/S1286-4579(01)01488-2