

Mechanisms and Biological Outcomes of Essential Oils as Functional Feed Additives in Laying Hens: A Systematic Review

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ABSTRACT

Essential oils are being investigated as phytogetic alternatives to antibiotic growth promoters in laying-hen nutrition because selected formulations contain compounds with antimicrobial, antioxidant, and immunomodulatory activities. The present study aimed to evaluate the mechanisms and biological outcomes of essential oils as functional feed additives in laying hens' diets, along with their effects on production performance and egg quality. The present systematic review followed the PRISMA 2020 framework. Peer-reviewed English-language studies published from 2020 to 2026 were identified through Scopus and Google Scholar databases. This period highlighted recent findings on the rising use of phytogetic feed additives in poultry nutrition. Twelve studies met the eligibility criteria and were included in the qualitative synthesis. Several studies reported changes in gut microbiota and intestinal morphology, improved antioxidant indicators, or modulation of immune-related variables. Selected studies also reported improvements in feed efficiency, egg production or egg mass, eggshell characteristics, and internal egg quality, whereas other outcomes were unchanged. The magnitude and consistency of responses varied by essential-oil composition, inclusion level, formulation or delivery method, age or laying phase, experimental duration, housing, and other study conditions. Overall, essential oils have potential as functional feed additives in laying hens, but the evidence does not support a uniform effect across all products. Their practical value depends on the specific formulation, dose, and application strategy.

Keywords: Antioxidant activity, Egg production, Essential oil, Gut microbiota, Immune response, Laying hen, Phytogetic feed additive

INTRODUCTION

Egg production is an important component of the global animal-protein supply, and maintaining laying performance requires attention to both productivity and hen health. In this review, physiological stability refers to the maintenance of oxidative status, immune-related responses, blood biochemical indicators, and intestinal health within ranges compatible with normal laying function. Antibiotic growth promoters can support feed efficiency and control enteric pathogens, but inappropriate or prolonged antibiotic use in poultry production is linked to antimicrobial resistance and residue-related food-safety concerns (Abou-Jaoudeh et al., 2024). Studies of poultry

production similarly identified antimicrobial resistance as a major issue associated with routine antibiotic use and emphasize the need for effective non-antibiotic strategies (Mehdi et al., 2018). Alternative-feed strategies were expected to maintain performance while supporting intestinal and immune function rather than serving as a simple replacement for antimicrobial inputs (Gadde et al., 2017).

Restrictions on routine antibiotic growth promoters have increased interest in alternative feed additives, including probiotics, prebiotics, organic acids, enzymes, phytogetic products, and essential oils (Acharya and Barsila, 2025). Phytogetic feed additives derived from herbs, spices, and other plant materials have been

investigated for antimicrobial, antioxidant, digestive, and immunomodulatory functions in poultry and other food-producing animals (Windisch et al., 2008; Hashemi and Davoodi, 2011). Phytochemicals have been suggested as antibiotic alternatives because of their effects on host defense and intestinal health (Lillehoj et al., 2018). Essential oils contain diverse bioactive constituents, and their biological activity depends on the plant source and chemical profile. Studies on non-ruminant nutrition indicated that essential oil activity depends on composition, dose, and interaction with the gastrointestinal environment (Zeng et al., 2015). In laying hen investigations, carvacrol and thymol from oregano preparations, cinnamaldehyde from cinnamon-based products, and terpinen-4-ol from tea tree oil have gained attention (Amerah and Ouwehand, 2016; Puvača et al., 2020). Feng et al. (2021) demonstrated that an oregano product containing carvacrol and thymol can affect intestinal and microbial responses in laying hens. Studies in post-peak laying hens have evaluated cinnamaldehyde combined with carvacrol or thymol, illustrating that specific combinations can cause different responses in intestinal health and egg quality (Wang et al., 2022).

Published studies in laying hens have reported effects of essential oil supplementation on intestinal morphology and microbiota, antioxidant status, immune-related variables, production traits, and egg quality; however, the findings have been inconsistent. Differences in essential oil type, bioactive composition, inclusion level, formulation, laying phase, and experimental conditions indicated that essential oils should not be regarded as a single intervention. Naim et al. (2024) examined essential oils' effects on egg production; however, the relationship between mechanistic responses and productive outcomes was not fully integrated, particularly given heterogeneity across studies. Therefore, a focused synthesis is needed to link biological mechanisms with production outcomes while distinguishing product-specific and study-specific responses. The present study aimed to evaluate the mechanisms and biological outcomes of essential oils as functional feed additives in laying hens, with emphasis on gut microbiota, intestinal function, antioxidant status, immune response, production performance, egg quality, and factors contributing to variation in responses across studies.

MATERIALS AND METHODS

This study used a systematic literature-review design and followed the Preferred Reporting Items for Systematic

Reviews and Meta-Analyses (PRISMA) 2020 guideline (Page et al., 2021). The purpose was to identify and qualitatively synthesize recent experimental evidence on essential oil interventions in laying hens; however, this study was not designed as a meta-analysis. Scopus and Google Scholar were used as the information sources. Scopus was selected to capture peer-reviewed literature indexed in a major international bibliographic database, while Google Scholar was used for broader scholarly searching to identify additional relevant articles. PubMed and Web of Science were not searched in the present review, and this restricted database coverage is acknowledged as a methodological limitation. Search terms included combinations of essential oil, phytogetic feed additive, laying hen, egg production, egg quality, intestinal health, gut microbiota, immune response, antioxidant status, blood parameters, digestibility, and gene expression. Boolean operators AND and OR were used to combine terms and refine retrieval. The publication window was limited to 2020-2026 to focus on recent evidence generated during increasing interest in phytogetic feed additives as alternatives to antibiotic growth promoters in poultry nutrition (References published before 2020 were used only for background, methodological context, or discussion and were not counted as included studies). Only peer-reviewed articles written in English were considered. The last literature search was conducted on 20 April 2026.

Peer-reviewed articles published in English between 2020 and 2026 that were conducted in laying hens, evaluated an essential oil or an essential-oil-based phytogetic additive, and reported at least one relevant outcome related to production performance, egg quality, gut health or microbiota, antioxidant status, immune response, blood parameters, digestive function, gene expression, or other physiological outcomes were considered eligible. Studies were excluded when they solely involved broiler chickens, quail, or other poultry without data on laying hens, lacked an essential-oil intervention, were reviews, conference abstracts, theses, dissertations, or other non-peer-reviewed sources, were not published in English, reported outcomes unrelated to the review questions, or failed to provide enough information for qualitative analysis. A total of 1,841 records were identified in Scopus (n = 1,341) and Google Scholar (n = 500). After duplicate removal, 1,711 records were screened by title; 1,321 records were excluded, leaving 390 records for title/abstract assessment. A further 345 records were excluded at this stage. Forty-five full-text articles were assessed for eligibility; 33 were

excluded, and 12 studies were included in the final qualitative synthesis. Figure 1 shows the selection pathway.

For each included study, extracted information included author and year, essential oil type, main bioactive compound (when reported), inclusion level or dose, experimental duration, age or laying phase, delivery or formulation method (when reported), parameters observed, and key findings. Age or laying phase was recorded because physiological stage can modify the context in which a response was observed. Study findings were grouped into biological mechanisms, production performance and egg quality, and factors explaining variation in responses. A qualitative synthesis was used

because the included studies differed substantially in essential oil source and composition, dose units, formulations, laying phase, duration, housing or challenge conditions, and outcome definitions. These differences did not provide a sufficiently homogeneous basis for a defensible pooled effect estimate. The reporting of findings without statistical pooling was informed by the Synthesis Without Meta-analysis (SWiM) guidance (Campbell et al., 2020). Table 1 summarizes the characteristics of the included studies, whereas Table 2 presents their key findings and outcome synthesis. References published before 2020 were used only for background, methodological context, or discussion and were not counted as included studies.

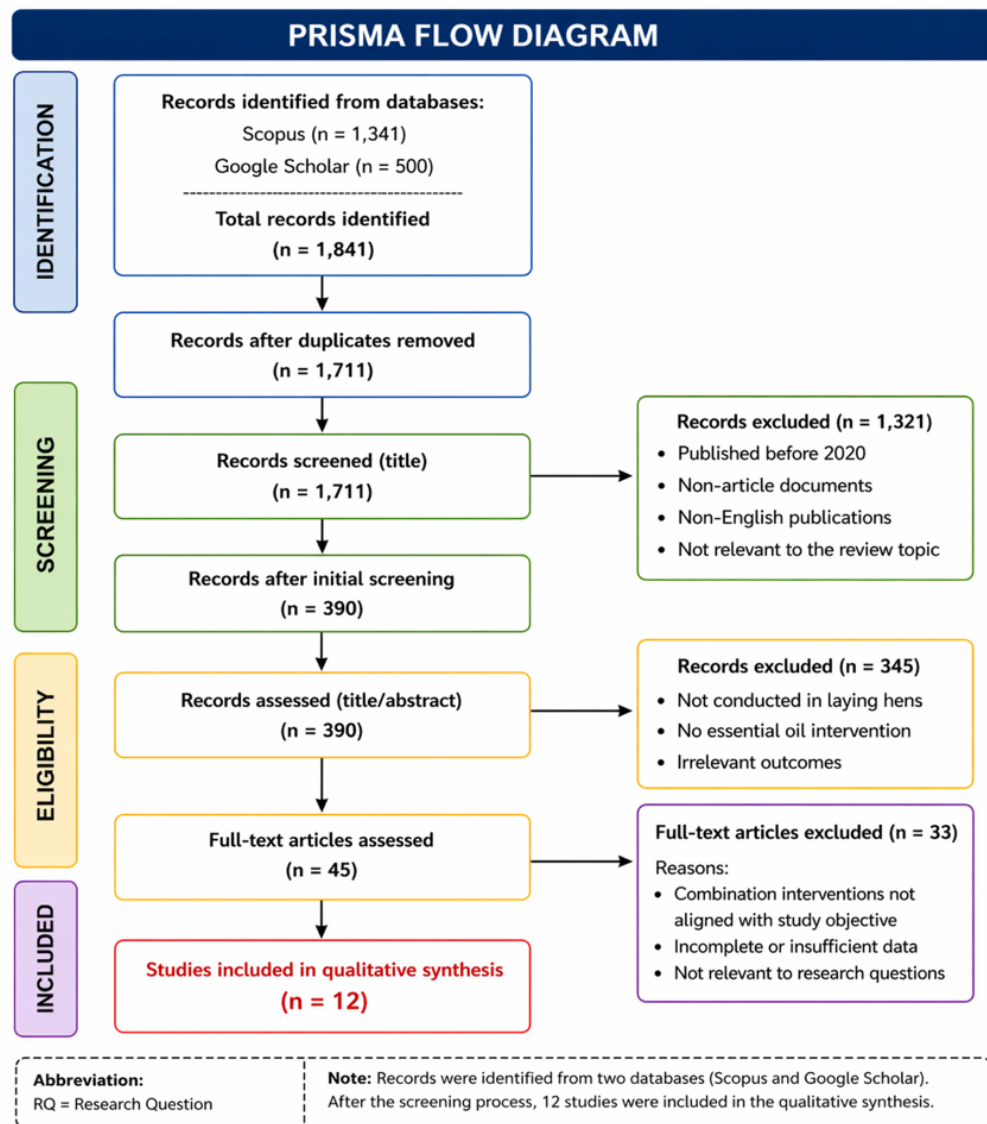


Figure 1. PRISMA flow diagram of the study selection process. The diagram was prepared by the authors based on the [PRISMA \(2020\)](#) guideline and summarizes article identification, screening, eligibility assessment, and final inclusion in the qualitative synthesis. Source: Authors' own illustration based on [PRISMA \(2020\)](#).

Table 1. Characteristics of studies on essential oils as functional feed additives in laying hens included in the present study (2020-2026)

Authors and year	Essential oil type	Main bioactive compound, if reported	Inclusion level or dose	Duration	Age or laying phase	Parameters observed
Marume et al. (2020)	<i>Citrullus lanatus</i> essential oil	Not reported	1 and 2 g/kg feed	8 weeks	18 weeks (point-of-lay)	Egg production, egg quality, yolk fatty acid profile, hematology, body weight, and tibia bone parameters
Feng et al. (2021)	Oregano essential oil (microencapsulated product)	Carvacrol and thymol	100, 200, and 400 mg/kg product	12 weeks	60 weeks (late phase)	Feed efficiency, egg quality, digestive enzymes, intestinal morphology, epithelial barrier, mucosal immune markers, and ileal microbiota
Ramirez et al. (2021)	Oregano essential oil (<i>Lippia organoides</i>)	Not reported	80 and 150 ppm	Study period June-December 2018; treatment duration not explicitly reported	70 weeks	Performance, egg quality, and intestinal morphometry
Xian et al. (2024)	Oregano oil	Not reported	25, 50, 75, and 100 mg/kg	100 days	55 weeks (late phase)	Egg quality, yolk nutrients, intestinal morphology, and cecal microbiota
Johnson et al. (2025)	Oregano essential oil (commercial product)	Not reported	275 mg/kg diet	16 weeks	24-40 weeks (early/peak phase)	Production, blood lipid profile, antioxidant capacity, liver-health indicators, egg quality, and yolk lipid peroxidation
Puvača et al. (2020)	Tea tree essential oil (<i>Melaleuca alternifolia</i>)	Terpinen-4-ol (40.0%), gamma-terpinene (23.0%), and alpha-terpinene (10.4%)	40 and 80 mg/kg	56 days	54 weeks at allocation; 56-day trial	Production performance, egg quality, eggshell traits, and yolk fatty acid profile
Xiao et al. (2022)	Essential oil blend	Carvacrol (2.5%), thyme-derived component (5%), and cinnamaldehyde (2.5%)	300 mg/kg	8 weeks	55-63 weeks (late phase)	Laying performance, egg quality, serum biochemical indices, antioxidant status, and cecal microbiota
Hanif et al. (2024)	Mineral mix-enriched essential oil	Not reported	0.25% in diet	6 weeks	31-37 weeks	Egg production, feed efficiency, antibody titer, physical and chemical egg quality, yolk antioxidant activity, and fatty acid profile
Abo Ghanima et al. (2020)	Rosemary essential oil or cinnamon essential oil	Not reported	300 mg/kg diet	48 weeks	28-76 weeks	Performance, egg quality, hematology, blood chemistry, immunity, antioxidant status, and housing-system effects
Laptev et al. (2021)	Essential oil-based phytobiotic, Intebio® (garlic, lemon, thyme, and eucalyptus oils)	Individual bioactive compounds not reported	90 g/ton feed	21 days before challenge; sampling at 1 and 7 days after inoculation; egg traits recorded for 27 days	346 days at study start; challenge at 367 days	Gene expression, blood biochemical and immunological variables, cecal microbiome, <i>Salmonella</i> challenge, and egg production
Reyer et al. (2026)	Phytogenic feed additive based mainly on thyme and star anise essential oils with quillaja bark	Not reported	150 mg/kg phytogenic supplement	4 weeks	68 weeks	Nutrient digestibility, egg quality, gene expression in pancreas, kidney, uterus, and follicles, and immune-related transcriptomics
Mus et al. (2026)	Oregano essential oil alone or combined with cinnamon or peppermint essential oil	Not reported	250 mg/kg oregano; or 125 mg/kg oregano + 125 mg/kg cinnamon; or 125 mg/kg oregano + 125 mg/kg peppermint	28 days	57 weeks	Intestinal microbiota, egg sensory and texture properties, fatty acid profile, and nutritional indices

Table 2. Key findings and outcome synthesis of included studies

Authors and year	Essential oil type	Inclusion level	Main outcome category	Key findings
Marume et al. (2020)	<i>Citrullus lanatus</i> essential oil	1 and 2 g/kg feed	Production; egg quality; hematology; bone	Egg mass and feed conversion ratio improved, as did albumen height and Haugh unit; selected yolk fatty acids, hematological values, and tibia parameters also changed. Total egg number was not significantly increased.
Feng et al. (2021)	Oregano essential oil	100, 200, and 400 mg/kg product	Gut function/microbiota; immune; production; eggshell	Feed conversion ratio, eggshell thickness, digestive-enzyme activity, intestinal morphology, epithelial-barrier markers, mucosal immune status, and ileal microbiota were affected; several favorable responses were strongest at 200 mg/kg.
Ramirez et al. (2021)	Oregano essential oil (<i>Lippia origanoides</i>)	80 and 150 ppm	Production; egg quality; intestinal morphometry	Both oregano treatments improved feed conversion and intestinal morphometry; 150 ppm produced the clearest improvements in egg production, egg mass, and internal and external egg-quality traits.
Xian et al. (2024)	Oregano oil	25, 50, 75, and 100 mg/kg	Egg quality; intestinal morphology; cecal microbiota	Oregano oil altered cecal microbiota and intestinal morphology and improved selected eggshell and yolk nutrient traits; 25 mg/kg produced the best overall response among the tested levels.
Johnson et al. (2025)	Oregano essential oil	275 mg/kg diet	Production; blood/liver; antioxidant; egg quality	Supplementation was associated with lower feed conversion ratio, changes in blood lipid profile, greater antioxidant capacity, lower lipid peroxidation, improved liver-health indicators, and selected improvements in internal egg quality.
Puvača et al. (2020)	Tea tree essential oil (<i>Melaleuca alternifolia</i>)	40 and 80 mg/kg	Production; eggshell; yolk fatty acids	Egg production and eggshell thickness improved in selected periods. Changes in the yolk fatty acid profile were directionally favorable but were not statistically significant overall.
Xiao et al. (2022)	Essential oil blend	300 mg/kg	Egg quality; serum biochemistry; antioxidant; microbiota	The blend increased shell-breaking strength and antioxidant indicators, altered serum biochemical indices, and shifted cecal microbial composition; production responses were more limited.
Hanif et al. (2024)	Mineral mix-enriched essential oil	0.25% in diet	Production; immunity; egg quality; yolk antioxidant	Egg production, feed efficiency, and yolk antioxidant activity increased. AI and ND antibody titers and yolk fatty acid profile were not significantly changed.
Abo Ghanima et al. (2020)	Rosemary essential oil or cinnamon essential oil	300 mg/kg diet	Production; egg quality; blood; immunity; antioxidant	Rosemary or cinnamon supplementation was associated with favorable changes in laying performance and selected egg-quality, blood, immune, and antioxidant measures. Housing system was evaluated concurrently.
Laptev et al. (2021)	Essential oil-based phytobiotic, Intebio®	90 g/ton feed	Gene expression; blood; microbiome; challenge; production	The phytobiotic modulated immune-related gene expression, blood biochemical and immunological variables, and cecal microbiome profiles during <i>Salmonella Enteritidis</i> challenge, with concurrent changes in egg-production traits.
Reyer et al. (2026)	Thyme/star-anise phytogetic feed additive with quillaja bark	150 mg/kg	Digestibility; transcriptomics; immune-related gene expression	Supplementation increased apparent ileal crude-protein digestibility and altered expression of genes related to antimicrobial activity, biomineralization, nutrient use, and immune responses.
Mus et al. (2026)	Oregano alone or with cinnamon/peppermint essential oils	250 mg/kg oregano; or 125 + 125 mg/kg combinations	Intestinal microbiota; egg composition; sensory/texture; fatty-acid indices	Oregano-based treatments reduced selected undesirable bacterial groups, increased lactic-acid bacteria, improved several fatty-acid-based nutritional indices, and did not produce undesirable sensory or texture changes in cooked eggs.

AI: Avian influenza; ND: Newcastle disease.

BIOLOGICAL MECHANISMS

The included studies identified intestinal function, microbiota, oxidative status, and immune regulation as recurrent biological domains, but the specific response depended on the intervention. [Feng et al. \(2021\)](#) found that a microencapsulated oregano essential oil product at 100-400 mg/kg affected digestive-enzyme activity, villus-to-crypt relationships, epithelial-barrier gene expression, mucosal immune markers, and ileal microbiota in 60-week-old late-phase hens. Several responses were most favorable at 200 mg/kg, suggesting the dose-response relationship is not linear. [Xian et al. \(2024\)](#) similarly reported that oregano oil at 25-100 mg/kg altered cecal microbial composition and intestinal morphology in 55-week-old hens, with 25 mg/kg yielding the most favorable overall response across egg production and intestinal health indicators. [Xiao et al. \(2022\)](#) observed that in late-laying hens, a 300 mg/kg essential oil blend enhanced total antioxidant capacity, boosted superoxide dismutase activity, altered specific serum biochemical indices, and modified the cecal microbiota. These findings support a connection between intestinal and oxidative responses, while also showing that not all microbiota-related and production endpoints changed in the same direction.

[Laptev et al. \(2021\)](#) reported that an essential oil-based phytobiotic containing garlic, lemon, thyme, and eucalyptus oils modulated immune-related gene expression, blood biochemical and immunological variables, and cecal microbiome profiles in hens infected by *Salmonella Enteritidis*. [Reyer et al. \(2026\)](#) found that a

thyme- and star-anise-based phytogetic additive containing quillaja bark enhanced apparent ileal crude-protein digestibility and induced transcriptomic changes in the pancreas, kidney, uterus, and follicles, involving genes related to antimicrobial activity and immune function.

Antioxidant responses were shown to be product-specific. [Johnson et al. \(2025\)](#) reported greater antioxidant capacity and lower lipid peroxidation with oregano essential oil, while [Abo Ghanima et al. \(2020\)](#) observed changes in antioxidant and blood parameters with rosemary or cinnamon essential oils. Reduced lipid peroxidation may indicate enhanced cellular integrity rather than a universal consequence of essential oils, aligning with findings from several studies suggesting that essential oil constituents can modulate oxidative and inflammatory mechanisms ([Bakkali et al., 2008](#); [Miguel, 2010](#)). Additionally, the antimicrobial efficacy of essential oils may be associated with the physicochemical properties of individual constituents and their interactions with microbial cellular structures ([Burt, 2004](#)). In poultry nutrition, proposed mechanisms include modulation of gut microbial ecology, digestive activity, and antioxidant or immune-related pathways ([Brenes and Roura, 2010](#)). These findings indicated that biological effects may include microbiota modulation, intestinal barrier support, oxidative balance, immune signaling, and systemic biochemical changes, each influenced by composition, dose, formulation, laying phase, and experimental conditions. These proposed mechanisms and their reported downstream outcomes are summarized in Figure 2.

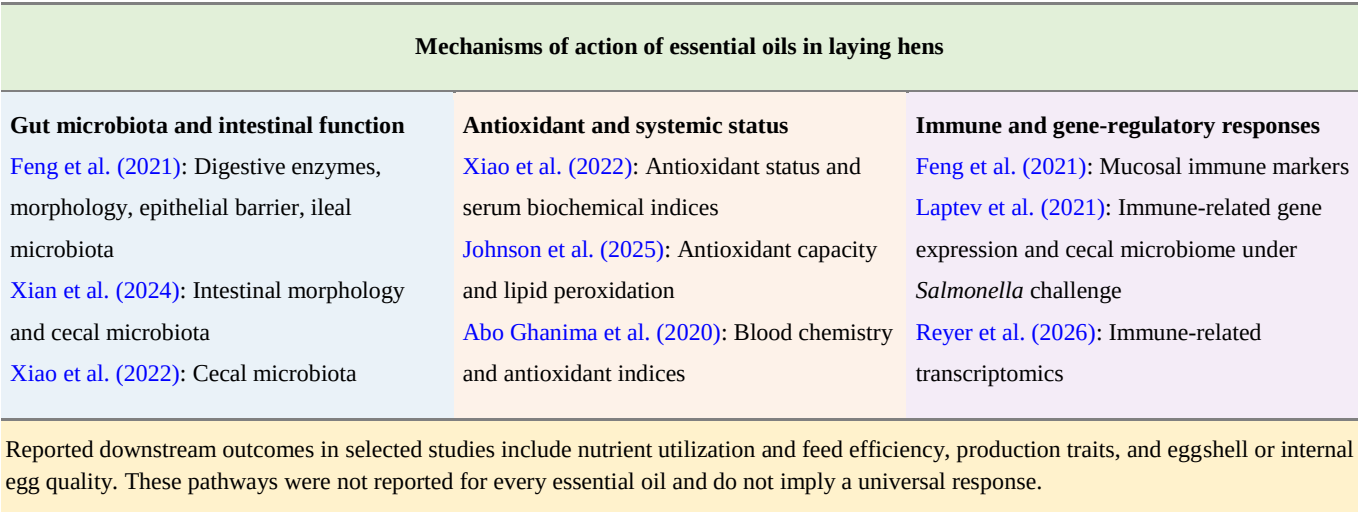


Figure 2. Proposed mechanisms by which essential oils influence biological and productive responses in laying hens. Essential oils may modulate gut microbiota, support intestinal morphology, enhance antioxidant defense, and regulate immune responses. These mechanisms may improve nutrient utilization, physiological stability, egg production, and egg quality, depending on essential oil composition, dose, and experimental conditions. Source: Authors' own illustration based on the synthesis of included studies.

PRODUCTION PERFORMANCE AND EGG QUALITY

Several included studies reported improvements in selected production traits after essential oil supplementation, although the magnitude and consistency of the response differed among products and experiments. [Marume et al. \(2020\)](#) found that administering 1 or 2 g/kg of *Citrullus lanatus* seed oil improved egg mass and feed conversion ratio in 18-week-old laying hens. However, total egg number did not increase significantly. [Ramirez et al. \(2021\)](#) reported improved feed conversion and intestinal morphometry with 80 or 150 ppm of *Lippia origanoides* essential oil. Notably, the 150 ppm treatment produced the most pronounced improvements in egg production, egg mass, and several internal and external egg-quality parameters.

Oregano-based supplementation yielded context-specific responses in other studies. [Feng et al. \(2021\)](#) reported a lower feed conversion ratio and greater eggshell thickness in late-phase hens, with the strongest responses at 200 mg/kg. [Johnson et al. \(2025\)](#) reported that administering 275 mg/kg of oregano essential oil from 24 to 40 weeks of age resulted in a lower feed conversion ratio, along with alterations in blood lipid profile, antioxidant capacity, liver health indicators, and internal egg quality. These findings suggested that production responses can occur alongside digestion, metabolism, or antioxidant activity; however, the mechanism applicable to each oregano formulation differed across studies.

Other essential oil sources produced more selective outcomes. Tea tree essential oil at 40 or 80 mg/kg improved egg production and eggshell thickness, whereas overall yolk fatty-acid changes were not statistically significant ([Puvača et al., 2020](#)). [Hanif et al. \(2024\)](#) discovered that enriching essential oil products with a 0.25% mineral mix enhanced egg production, feed efficiency, and yolk antioxidant activity. However, this product did not significantly affect antibody titers for avian influenza or Newcastle disease, nor did it alter the yolk fatty-acid profile. [Abo Ghanima et al. \(2020\)](#) observed improvements in laying performance, specific egg quality traits, blood parameters, immune response, and antioxidant markers when using rosemary or cinnamon essential oils at 300 mg/kg of diet, and also evaluated floor and cage housing systems.

The effects on egg quality varied by trait. Albumen height and Haugh unit increased with the addition of *Citrullus lanatus* ([Marume et al., 2020](#)), eggshell thickness

or strength improved with several oregano and tea tree oils ([Puvača et al., 2020](#); [Feng et al., 2021](#); [Xiao et al., 2022](#)), and [Xian et al. \(2024\)](#) reported changes in eggshell traits and yolk nutrients with oregano oil. The available evidence supports the conclusion that selected essential-oil formulations can improve specific production or egg-quality endpoints, rather than the broader claim that all essential oils improve all production traits.

VARIATION IN ESSENTIAL OIL RESPONSES

Differences across studies were closely related to chemical composition and formulation. Oregano-based products were the most commonly represented interventions in these 12 studies; however, they were not chemically or technologically identical. [Feng et al. \(2021\)](#) employed a microencapsulated oregano product containing carvacrol and thymol, while other oregano studies utilized different commercial formulations or *Lippia origanoides* oil ([Ramirez et al., 2021](#)). Tea tree oil contains terpinen-4-ol, gamma-terpinene, and alpha-terpinene ([Puvača et al., 2020](#)), while [Xiao et al. \(2022\)](#) used a mixture containing carvacrol and cinnamaldehyde with a thyme-derived component. Variation in chemical composition, stability, and formulation is a recognized source of inconsistent responses when essential oils are used as feed additives ([Dajić Stevanović et al., 2018](#)).

Additionally, dose can influence response variability, but current findings did not indicate a uniform optimal dose for all products. [Feng et al. \(2021\)](#) found that the most pronounced intestinal and production responses occurred at 200 mg/kg compared with 100 mg/kg and 400 mg/kg. In a study by [Xian et al. \(2024\)](#), the lowest tested level, 25 mg/kg, produced the best overall response compared with the higher tested levels of 50, 75, and 100 mg/kg. Thus, lower or intermediate tested doses sometimes performed more consistently than the highest tested dose, but this pattern cannot be generalized across essential oils because dose units, product concentration, and bioactive composition differed among studies.

Formulation and delivery further complicated comparison across the studies. Most included trials administered the essential oil via feed; however, the products included individual oils, oil mixtures, a microencapsulated oregano formulation, a mineral-enriched preparation, and a phytobiotic derived from essential oils. Encapsulation can improve the physical stability of essential oils and alter their release in the gastrointestinal tract; consequently, nominal inclusion

levels are not always directly comparable between encapsulated and unencapsulated formulations (Fontana et al., 2025). Mus et al. (2026) directly compared oregano essential oil alone with oregano-cinnamon and oregano-peppermint combinations in 57-week-old hens and observed treatment-specific changes in intestinal microbiota and egg fatty-acid indices without undesirable sensory effects. Xiao et al. (2022) evaluated the effects of a different essential oil mixture. These studies indicated that combining multiple oils did not automatically mean the mixture was synergistic.

Age and laying phase were another source of heterogeneity among the included studies. The included studies ranged from 18-week-old (Marume et al., 2020) to 70-week-old laying hens (Ramirez et al., 2021), with late-phase chickens evaluated in the studies of Feng et al. (2021), Xiao et al. (2022), and Xian et al. (2024) and 68-week-old laying hens in the study conducted by Reyer et al. (2026). The physiological priorities of early, peak, post-peak, and late laying differ, so age was treated as a study-level modifier rather than ignored during synthesis.

Experimental settings differed across the included studies. Abo Ghanima et al. (2020) explicitly evaluated floor and cage housing with rosemary or cinnamon

essential oils, whereas the remaining studies did not uniformly report housing, ambient conditions, or heat-stress status. Experiment durations ranged from 28 days in the study by Mus et al. (2026) to 48 weeks in the study conducted by Abo Ghanima et al. (2020). The evaluated factors spanned a broad range, including standard performance, egg-quality metrics, microbiota sequencing, blood indices, gene expression, and transcriptomic analyses. Consequently, inconsistent findings may reflect differences in measured factors, sampling times, and physiological or environmental conditions.

Across the included studies, oregano-based essential oils were most frequently associated with positive responses in gut function or microbiota, feed efficiency, antioxidant status, and selected egg-quality traits. This pattern should be interpreted cautiously because oregano-based studies were also the most numerous and differed in inclusion level, duration, laying phase, formulation, and measured outcomes. The qualitative design of the present review does not permit ranking oregano as universally superior to other essential oils. The main product- and study-level factors contributing to response variability are summarized in Figure 3.

Factors influencing variation in essential oil responses in laying hens		
Composition and bioactive profile Oregano preparations, tea tree oil, rosemary/cinnamon oils, single oils, blends, and phytobiotics differed in chemical profile.	Inclusion level Doses varied within and between products, with oregano studies using 25-400 mg/kg and other studies using g/kg, ppm, %, or g/ton units.	Formulation and delivery Most studies used feed inclusion; formulations included microencapsulated oregano, blends, mineral-enriched products, and a phytobiotic.
Age or laying phase Hens ranged from point-of-lay (18 weeks) to late/post-peak phases (55-70 weeks or older), creating different physiological contexts.	Housing and environment Housing, environmental conditions, and heat-stress status were not uniformly reported.	Duration and outcome measurement Experimental duration ranged from 28 days to 48 weeks, and studies measured different biological, production, egg-quality, blood, and omics endpoints.
Interpretation: variability across studies reflects interacting product- and study-level factors; the qualitative synthesis does not establish a universal ranking of essential oils.		

Figure 3. Factors contributing to variability in essential oil responses in laying hens. Differences in chemical composition, inclusion level, delivery method, laying phase, housing system, and environmental condition may influence the consistency and magnitude of biological and production outcomes across studies. Source: Authors' own illustration based on the synthesis of included studies.

CONCLUSION

Twelve studies were included and indicated that specific essential oil formulations can affect gut microbiota and

intestinal health, antioxidant levels, immune responses, systemic biochemical markers, production performance, and egg quality in laying hens. Oregano-based preparations were the most common intervention and were

most often linked to positive effects across outcomes, especially gut function, feed efficiency, antioxidant status, and specific egg-quality traits. The present findings should not be interpreted as definitive evidence that oregano is the most effective essential oil overall, since the review was qualitative and did not include a meta-analysis. Responses to essential oil formulations differed across studies due to variation in chemical composition, dose, formulation or delivery method, age or laying phase, duration, housing, and experimental conditions. The present study was constrained by reliance on Scopus and Google Scholar databases, heterogeneity in experimental design and outcome measurement, and incomplete reporting of bioactive composition or treatment details across studies. Future studies should report essential oil composition and delivery more consistently, use clearly defined laying phases and comparable outcome measures, and broaden database coverage.

DECLARATIONS

Authors' contributions

Luky Amalta contributed to the study conception and design, literature search, screening, data extraction, qualitative synthesis, and manuscript drafting. Osfar Sjoifan, Eko Widodo, Heli Tistiana, Ita Wahju Nursita, Dita Novarina Gunawati, and Cindy Audina Damayanti contributed to methodological review, interpretation of the qualitative synthesis, critical revision, and improvement of intellectual content. All authors read and approved the final edition of the manuscript.

Availability of data and materials

All data analyzed in this review were obtained from previously published articles cited in the manuscript.

Competing interests

The authors declare no competing interests.

Ethical considerations

The authors confirm that this manuscript is original and is not currently under consideration for publication elsewhere. The authors accept full responsibility for the originality of the content, accuracy of the data extraction, and the final edited version of the manuscript. Since this study was a systematic literature review and did not involve human participants or live animals, ethical approval and informed consent were not required. The authors did not use any AI tools to conduct or prepare this study. The authors accept full responsibility for the

originality and the integrity of the present review manuscript, before submission and at publication time.

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