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Essential Oils as an Alternative to Antibiotics to Reduce the Incidence and Severity of Necrotic Enteritis in Broiler Chickens: A Short Review

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Abstract

Due to the removal of antibiotic growth promoters (AGPs) and consumer pressure for antibiotic-free (ABF) or no antibiotics ever (NAE) poultry production, there is a need for sustainable alternatives to prevent disease in commercial poultry operations. Without AGPs, there has been a rise in diseases that were traditionally controlled by subtherapeutic levels of antibiotics in the diet. This has impacted the health of commercial poultry and has been a significant cost to poultry producers. To mitigate this, the industry has started to investigate alternatives to antibiotics to treat these forthcoming health issues, such as necrotic enteritis (NE). NE is an enteric disease caused by an over proliferation of toxigenic *Clostridium perfringens* (CP) in the gastrointestinal tract. Although CP is a commensal in the avian intestinal tract, dysbiosis caused by inflammation and impaired intestinal integrity facilitates uncontrolled replication of CP. Infectious agents, such as *Eimeria maxima*, appear to be a predominant predisposing factor that promotes NE. However, non-infectious stressors, including dietary changes, have also been associated with NE to some degree. As a result of increased pressure to restrict the use of antibiotics, there is a need for research evaluating the efficacy of alternatives, such as plant-derived essential oils, as potential tools to mitigate NE in commercial poultry flocks. The aim of this study is to review the effects of essen-

tial oils as an alternative to antibiotics to reduce the incidence and severity of necrotic enteritis in broiler chickens.

Keywords

Necrotic Enteritis, Essential Oils, Chickens, *Clostridium perfringens*, Alternatives to Antibiotics

1. Introduction

Over the last few hundred years, humans have influenced the evolution of multiple animal species and different ecosystems involved in animal production [1]. This approach has led to the genetic change of domestic animals and has undoubtedly been driven by agriculture. The most important genetic changes in poultry production have occurred in the previous 60 years. Modern broiler chickens are likely the clearest example of these genetic improvements. Newborn chicks grow 31% (55 g/bird) on day one, and 5902% (2521 g/bird) on day 35 [2]. Intensive genetic selection, diet, health, and management initiatives have led to these achievements. Nonetheless, maintaining the integrity of the gastrointestinal tract (GIT), the primary organ responsible for digestion and nutritional absorption, is critical for production. Because feed conversion accounts for over 70% of the cost of production in poultry and animal enterprises, subclinical coccidiosis and necrotic enteritis in chickens are more costly than acute infections (**Figure 1**). As the growing period of broilers shortens and feed efficiency improves, so do health and nutrition programs. Therefore, increasing the importance of a well develop intestinal epithelia and a balance host-diet-microbiota interaction that influences gut health and overall health and productivity.

Essential oils (EO) have received a lot of attention as nutraceuticals in livestock production in recent years, primarily as an alternative to antibiotic growth promoters (AGPs) worldwide. Essential oils are secondary metabolites derived from various plants with well-documented antibacterial, antiviral, antifungal, antioxidant, digestive stimulant, and immunomodulatory properties [3] [4] [5] [6] [7]. Some EO are used in conjunction with other phytochemicals to improve poultry performance [8]. As a result, EO have played a critical role in reducing the increased incidence of coccidiosis and necrotic enteritis (NE) caused by the removal of ionophores and AGPs. Because NE is a multifactorial disease caused by Eimeria spp. and *Clostridium perfringens*, EO is frequently combined with other strategic products such as probiotics, prebiotics, organic acids, and enzymes to modulate the intestinal microbiota and immune system of birds [9] [10] [11]. The aim of this study is to review the effects of essential oils as an alternative to antibiotics to reduce the incidence and severity of necrotic enteritis in broiler chickens.

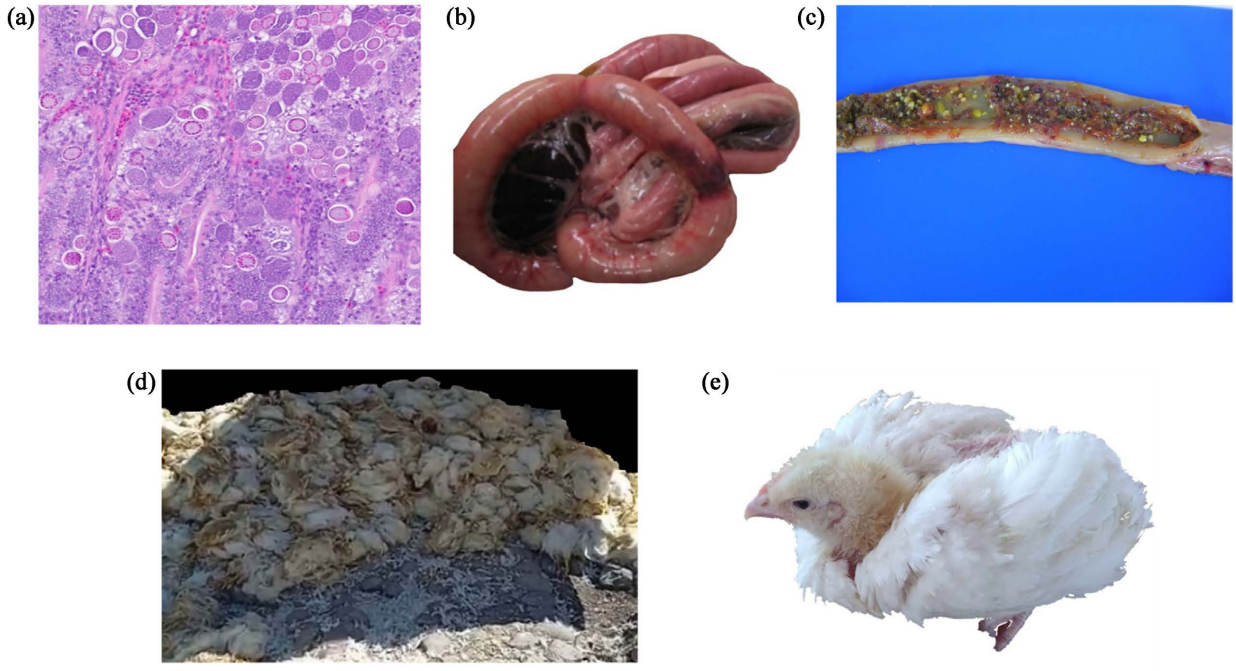

Figure 1. Even though necrotic enteritis is a multifactorial disease, *Eimeria maxima* has been considered a primary pathogen in the clinical and subclinical outbreaks of necrotic enteritis. (a) Mucosal and submucosal jejunum with infiltration of inflammatory cells, ulceration, necrosis, and the presence of *E. maxima* oocysts. Hematoxylin and eosin staining. NE causes macroscopic (b) ballooning of intestines and (c) extensive sloughing of the intestinal mucosa and hemorrhaging. (d) While clinical and acute outbreaks can induce severe mortality, the global economic losses are due to (e) subclinical necrotic enteritis, affecting all performance parameters. (Images courtesy of Dr. Victor M. Petrone-Garcia and Created with BioRender.com).

2. Importance of GIT Health in Poultry

The GIT is home to a varied microbial community known as gut microbiota [12], outnumbering somatic cells by tenfold, with 300,000 genes compared to 23,000 genes in chickens [13] [14]. The enteric nervous system (ENS) has approximately one hundred million neurons and is referred to as the "second brain" of metazoans because of its importance in digestion [15]. Approximately 80 percent of the immune cells in the body are found in the gut-associated lymphoid tissue (GALT). The Bursa of Fabricius, a lymphoid organ that is critical for B-lymphocyte growth and proliferation in avian species, is a component of the GALT [16]. As an astonishment, the GALT comprises 80 percent of the plasma cells that are responsible for the production of secretory immunoglobulin A (IgA), the far more prevalent immunoglobulin [17].

A range of physiological processes, including secretion, absorption, digestion, and gut motility, are mediated by enteroendocrine cells (EECs), which also play a role in the etiology of intestinal mucosa atrophy and malignancies, both within and beyond the GIT [18]. Gastrin, secretin, cholecystokinin, insulin, and glucagon were among the first GIT hormones to be discovered in humans [19]. The discovery of more than 50 gut hormones and bioactive peptides today confirms that the gut is the body's largest endocrine organ, performing an extensive spec-

trum of endocrinological, neuroendocrine, autocrine, and paracrine functions, as well as a variety of other roles [20]. Enterochromaffin cells, a subset of several EECs, produce 90% of the neurotransmitter serotonin (5-hydroxytryptamine), which plays multiple biological roles in temperament, perception, reproduction, vasodilation, gut motility, wound healing, and vasoconstriction [21]. Surprisingly, the gut microbiome modulates serotonin and other EEC-produced mood neurotransmitters like dopamine, oxytocin, and endorphins [22] [23] [24]. Published research have shown that in humans, illnesses of the brain (such as schizophrenia, depression, Alzheimer's disease, Parkinson's disease, and autism) are associated with the kind of microbiota prevalent in the GIT [25] [26]. The cliché "gut instincts" holds true in this case [27].

For more than a century, Eli Metchnikoff, the Nobel Prize-winning father of innate immunity, offered the breakthrough idea of ingesting live bacteria to boost health by modifying the intestinal microbiota [28] [29]. Antibiotic resistance in bacteria (sometimes known as "superbugs") is a major problem in medicine and agriculture around the world. As the number of antibiotic-resistant bacteria grows, this concept is becoming increasingly relevant [30]. According to recent research, nutritional approaches may be effective alternatives to antibiotics in some cases [31] [32] [33] [34]. In addition to increasing animal health, welfare, and production, boosting disease resistance in antibiotic-free animals is an important task in enhancing food safety. The gut microbiota influences the host's biology, metabolism, nutrition, immunity, and neuroendocrine system [35] [36]. Short-chain fatty acids, gastrointestinal hormones, enteroendocrine and immune cells all play a role in these effects [37]. The enteric nervous system and hormonal networks control GIT motility, which is impaired in functional GIT diseases [38]. The neuroendocrine network that connects the brain, the ENS, gut microbiota, and the GALT has a significant impact on the delicate intestinal epithelial barrier [39] [40]. This barrier, which consists of a single layer of enterocytes with tight intercellular junctions, regulates the balance of tolerance and immunity to non-self antigens [41]. Hence, gut integrity is critical in maintaining a healthy balance of health and disease [42]. To keep the system in survival mode, chronic stress and chronic intestinal inflammation divert significant biological resources away from development and reproduction. Perhaps a more comprehensive definition of "gut health" should include the harmonious interaction of the microbiota-brain-gut axis [35] [43] [44].

All biological and physiological processes maintain the various microbiomes that live on mucosal surfaces in balance [45]. Dysbiosis (loss of symmetry of the GIT microbiota) leads to loss of intestinal integrity [46]. Dietary ingredients and the viscosity of gut contents influence microbes in the small intestine [47]. Animal producers who have eliminated antibiotics from their production systems may use a combination of alternative products, improved management methods, stringent biosecurity, and successful immunization programs to achieve their health and productivity goals. However, chronic stress and persistent inflamma-

tion still harm modern animal production operations. Any source of chronic stress, whether biological, physical, chemical, toxic, or psychological, will cause oxidative stress and, if unabated, chronic intestinal and systemic inflammation [48] [49] [50]. Chronic intestinal and systemic inflammation opens up the gut to opportunistic bacteria such as *C. perfringens*.

2.1. *Clostridium* spp. in the GIT

To maintain gut homeostasis, a complicated mutualistic symbiosis maintains the host-microbiota connection [51]. Commensal Clostridia (Class) in the Firmicutes (Phylum) make up a large proportion of the gut microbiota [52]. Clostridial spp. begin colonizing the intestine at hatch, subsist near intestinal cells, and play an important role in altering gut physiology and immunology [52]. *Clostridium* (Genus) contains over one hundred beneficial species, and only a few are pathogenic [53]. They represent the most significant butyric acid-producing organisms in the GIT [53]. Commensal Clostridia play an active role in maintaining overall gut function [52]. Hence, distinguishing beneficial Clostridial species from potentially virulent ones, like *Clostridium perfringens,* is critical [54]. Clostridial cluster IV contributes to up to twenty percent of bacteria present in humans [55]. *Clostridium* clusters XIVa and IV members consistently decreased in patients with gut inflammation [56]. This implies that these organisms are vital to gastrointestinal homeostasis [54]. Clostridiales (Order) also increased mucosal tolerance to commensal microbiota by boosting IL-10 and transforming growth factor-beta expression levels in the gut [57]. Furthermore, Clostridiales such as *Ruminococcus* spp., *Faecalibacterium* spp., and *Lachnospiraceae* spp. are the remarkable bacteria that produce butyrate [58] [59], inducing profound physiological reactions in the gut [60]. *Clostridium* cluster strains IV and XIVa are great inducers of T-regulatory cells and constitute a novel therapeutic alternative for intestinal inflammatory diseases [61]. Interestingly, probiotics have been demonstrated to cause significant alterations in butyrate and other key SCFA, which have a considerable impact on gut physiology and immunology [62]-[67]. Commensal *Clostridium* bacteria clearly are vital in gut homeostasis [52]. However, commensal Clostridial spp. such as *C. perfringens* rapidly proliferate when the broiler's epithelium is damaged [68]. There are predisposing factors that increase *C. perfringens* overgrowth such as nutritional components [69] and coinfections with *Salmonella* spp. [70] or *Eimeria* spp. [10] (**Figure 2**). Mucin-2 is the most abundant mucin that is secreted by intestinal epithelial cells [71]. *Eimeria* spp. have been shown to have an effect on the relative mucin secretion in each area of the GIT [72].

2.2. Necrotic Enteritis

Necrotic enteritis (NE) is caused by the ubiquitous bacterium *C. perfringens*. *C. perfringens* is an anaerobic, Gram-positive, endospore-forming, nonmotile, bacterium that can survive and persist in harsh environmental conditions. As the

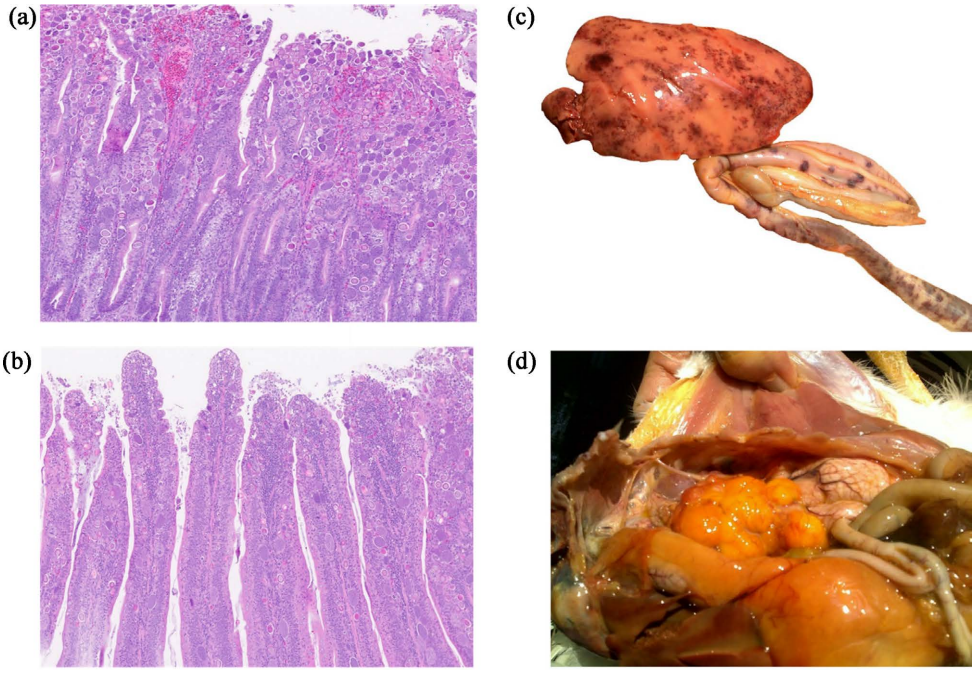

Figure 2. (a) Mucosal and submucosal jejunum with infiltration of inflammatory cells, ulceration, necrosis, and the presence of *E. maxima* oocysts. Hematoxylin and eosin staining. (b) Mucosal and submucosal duodenum with infiltration of inflammatory cells, ulceration, necrosis and the presence of *Eimeria acervulina* oocysts. Hematoxylin and eosin staining. (c) Duodenum and jejunum of a layer hen with necrotic enteritis. The liver shows areas of necrosis and hemorrhages due to liver bacterial translocation and chronic systemic inflammation. (d) Oviduct and ovary from the same layer hen showing hemorrhages and inflammation. (Images courtesy of Dr. Victor M. Petrone-Garcia and Created with BioRender.com).

chicken industry has reduced its usage of antibiotics, NE in both its clinical and subclinical forms have become a significant health, welfare, and performance problem [73]. Because NE is a complex disease, that usually includes a co-infection of Eimeria spp. and *C. perfringens*, management without antibiotics requires sustainable alternative prophylactic or therapeutic strategies [11]. The total global economic cost of NE was assessed to be more than $2 billion dollars in 2000 [74]. The number of broiler chickens produced increased from 14.38 billion in 2000 to approximately 33 billion in 2020 worldwide [75]. It was estimated that necrotic enteritis cost the United States poultry industry $6 billion annually [76].

C. perfringens is grouped into seven toxigenic categories (A-G) producing over twenty toxins [77]. The poultry industry is particularly interested in *C. perfringens* types A, C, and G [78] [79]. In this process, *C. perfringens* releases enzymes that break down host tissue, causing more tissue damage, inflammation, and disruption of the intestinal ecology, causing dysbiosis [80] [81] [82]. These *C. perfringens* strains produce pathogenic toxins including NetB toxin, which has been identified as a major factor associated with NE in broilers [83] (**Figure 3**). Field outbreaks of NE had one *C. perfringens* clone prevalent in the intestines of all infected birds, rather than the variety of strains found in healthy bird

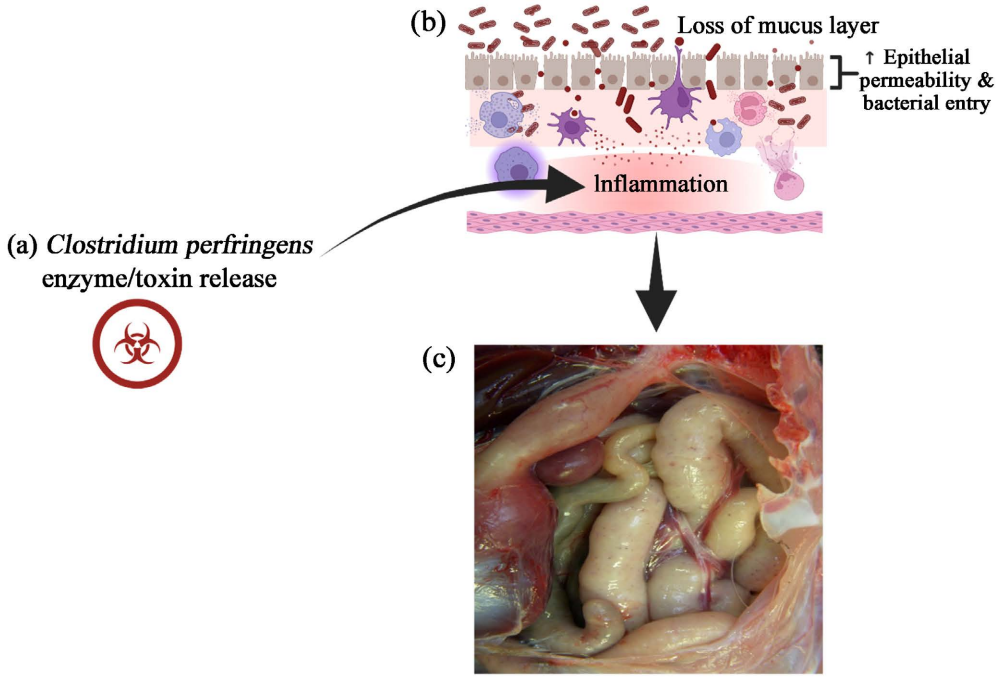

Figure 3. *C. perfringens* types A, C, and G release (a) enzymes that break down host tissue causing (b) inflammation, and disruption of the intestinal ecology, resulting in dysbiosis. NetB toxin, which has been established as a significant contributor in NE in broilers, is present in these lethal *C. perfringens* strains. Additionally, some *C. perfringens* strains have shown to produce collagenolytic enzymes that induce an initial pathological change in the enterocytes, contributing to the development of NE. (c) Lesions caused by *C. perfringens* proliferation and toxin production can be observed macroscopically. (Images courtesy of Dr. Victor M. Petrone-Garcia and Created with BioRender.com).

intestines. A single dominant *C. perfringens* strain associated with NE may be due to bacteriocin production [83]. Intestinal *C. perfringens* overgrowth has been linked to intestinal mucosa injury, low pH, coccidiosis, nutritional factors, stress, and immunosuppression [83] [84] [85]. Several investigators have evaluated different alternatives to reduce NE such as probiotics, prebiotics, symbiotic, and organic acids [9] [86] [87] [88].

C. perfringens infection alone is not enough to cause necrotic enteritis in broiler chickens. Predisposing factors are a key player in creating the right environment for the proliferation of virulent *C. perfringens*, producing disease [89]. These predisposing factors can include, but are not limited to, feed ingredients [90], coccidiosis caused by *Eimeria* spp. *[91]*, environmental stressors [92], and exposure to *Salmonella* spp. [70].

2.3. Alternatives to Antibiotics to Control NE

Due to the removal of AGPs and the shift to antibiotic free production systems, research investigating antibiotic alternatives have been on the rise. The incidence of Clostridial-related diseases, including NE, increased with implementation of AGP bans [93]. In an antibiotic-limited or antibiotic-free era, natural alternatives to optimize intestinal health and improve animal wellbeing and perfor-

mance are desperately needed. Probiotics are live, beneficial microorganisms that have been shown reduce colonization by enteric pathogens [94] [95] [96]. Direct-fed microbials [97], prebiotics [98] [99] [100], organic acids [101], plant extracts [102], essential oils [1] [103], and trace minerals [104] can help to improve intestinal microbial balance, metabolism, and gut integrity. Phytogenics have remarkable antioxidant, anti-inflammatory, antibacterial, and barrier integrity-enhancing assets. For example, supplementation with curcumin, a component in tumeric, reduced the severity of necrotic enteritis [105], salmonellosis [102] [105], and aflatoxicosis [106] in broiler chickens as well as coccidiosis in Leghorn chickens [107]. Additional investigations regarding phytogenics, specifically EOs and gut health are described below.

2.4. Essential Oils

Essential oils (EOs) are a derivative of plants. Revered for their medicinal properties, EOs are natural, volatile compounds usually associated with a strong odor [108]. The mode of action(s) of EOs has been extensively reviewed [109]. EOs inhibit *in vitro* proliferation of Gram-negative and Gram-positive bacteria by increasing membrane permeability of the cell wall and mitochondrial membranes [109]. Antimicrobial efficiency of EOs is impacted by the compound's hydrophobicity [110]. *In vitro* studies show evidence that when compared to standard antimicrobial agents, EOs have a similar effect on the growth inhibition of *C. perfringens* [111].

EOs have been increasingly popular as feed additives over the last two decades [112] due to their antibacterial, antiviral, antifungal, anti-inflammatory, immunomodulatory, epithelial barrier, microbiota modulation, production performance and anti-hyperlipidemic properties [4] [5] [6] [7] [8] [113] [114] (**Figure 4**). Lippia origanoides or Thymus Vulgaris L. contains up to 3% essential oil containing monoterpenes, mainly thymol and its phenol isomer carvacrol. Phenolics in essential oil such as caffeic acid, p-cymene-2,3-diol and some biphenylic and flavonoid compounds like flavonoid glycosides, flavonoid aglycones are assumed to contribute various beneficial effects and have been used as a feed additive in poultry diets without adverse effects [115]. However, one of the most remarkable bioactive properties of EOs is their anti-oxidant effect, which prevents lipid peroxidation of the cell membrane and mitochondrial membrane phospholipids, as well as the denaturalization of proteins and DNA, thereby preventing multiple organ failure and other diseases [3] [116].

Some studies found in the literature using EOs instead of antibiotics have shown to reduce the severity of NE through the demonstrated bactericidal activity of EOs against *C. perfringens* [111] [117]. Other recent studies have revealed a reduction in NE-induced intestinal damage [10] [118] [119] [120] [121], reduction in mortality associated with NE [122] [123], regulation of the intestinal microbial communities [124] [125] [126], modulation of short-chain fatty acids profiles [84] [127], improvement of the morphometric and barrier functions

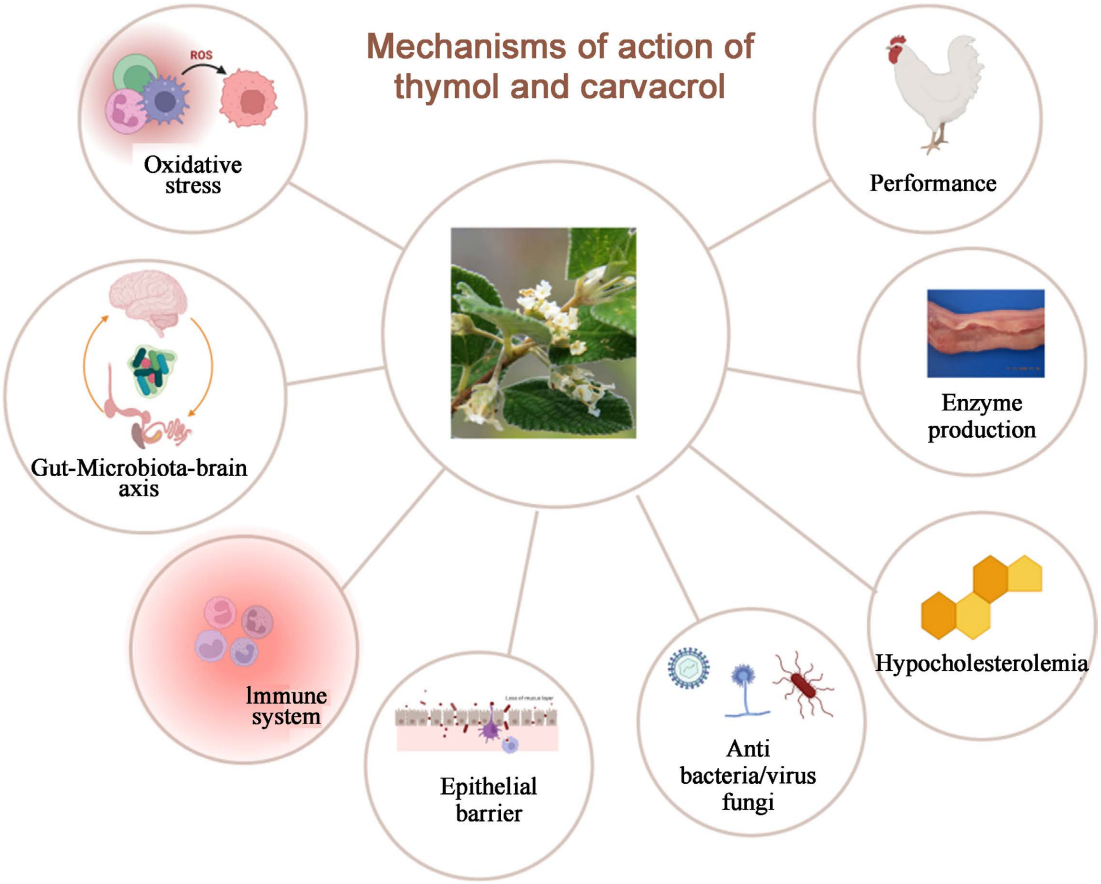

Figure 4. Carvacrol and thymol are two of the most abundant essential oils present in *Lippia origanoides*. Both essential oils have been extensively studied due to their anti-oxidant, gut microbiota modulation, immunoregulation, epithelial barrier, antifungal, antimicrobial, antiviral, anthelminthic, hypocholesterolemia, appetite stimulant and increased pancreatic enzyme production, and improving performance properties. (Images courtesy of Dr. Victor M. Petrone-Garcia and Created with BioRender.com).

[123] [128], as well as, reduction of oocyst counts [129] [130] and dysbacteriosis [131] [132]. EOs, especially thymol and carvacrol stimulate enzyme secretion and improve digestion, and several studies have shown a substantial impact of EOs on performance parameters and intestinal lesion scores in broiler chickens under different NE models (**Table 1**).

EOs have been shown to alter the host's immune response. Dietary inclusion of carvacrol, cinnamaldehyde or oleoresin altered gene expression of intestinal intraepithelial lymphocytes, with dietary oleoresin having the greatest effect on transcriptional regulation [133]. Furthermore, EOs limited pro-inflammatory cytokine production related to *C. perfringens* or *Eimeria* spp. challenge [134]. Pathogenicity of *C. perfringens* was modulated by the inclusion of EOs (25% carvacrol, 25% thymol; 120 mg/kg) in the diet which downregulated in vivo expression of *C. perfringens* virulence factors: VF 0073-ClpE, VF0124-LPS, and VF0350-BSH [126]. The altered host ileal microbiome composition and *C. perfringens* virulence factor expression was likely reduced intestinal lesion scores and mortality in broiler chickens [126]. The direct or indirect changes in the gut

Table 1. Impact of essential oils (EOs) on performance parameters and intestinal lesion score in broiler chickens under different necrotic enteritis (NE) models.

Essential oils/Source	Dietary inclusion	Performance parameter	NE lesion score	References
Citrus, oregano and annase EOs	1 g/kg of feed	Reduction in mortality (26% PC* vs 8% EOs).	1.33 vs 0.58 (PC vs EOs).	[120]
Ginger oil and carvacrol	1.5 g/kg of feed	EOs improve BW in 100 g compared to the PC.	3.0 vs 2.3 (PC vs EOs).	[135]
Capsicum oleoresin and turmeric oleoresin	4 mg of each oleoresin per kg of feed.	Improve BW ($P < 0.05$).	2.8 vs 1.2 (PC vs EOs).	[136]
Thymol and carvacrol	0, 60, 120 or 240 mg/kg of feed	EO linearly reduce FCR ($P = 0.056$).	1.5 vs <0.5 (PC vs EOs).	[134]
Thymol and carvacrol	120 mg/kg of feed	Reduction in mortality (20% PC vs 4% EOs).	>2 vs <1 (PC vs EOs).	[137]
Thyme (thymol) and clove (eugenol)	Combination: Thyme 2.5 g/kg and Clove 1.25 g/kg of feed	EOs improve BWG in 200 g compared to PC. FCR 2.84 PC vs 1.88 EOs.	3.0 vs 1.0 (PC vs Eos).	[117]
Peppermint oil	0.5 or 0.25 ml/ml of water	Reduction in mortality (55% PC vs 10% EOs). Improve BWG in 41 g	ND[€]	[138]
Thymol and carvacrol	120 mg/kg of feed	Reduction in mortality (20% PC vs 4% EOs).	>2 vs <1 (PC vs EOs).	[122]
Garlic nanohydrogel	100, 200, 300 or 400 mg/kg	EOs increase BWG 181 g, 367 g and 588 g (200, 300 and 400 mg/kg).	>2 vs <1 (PC vs 400 mg/kg EOs).	[139]
Thyme, savory, peppermint and black pepper	0.5, 1 or 2 g/kg of feed	Improvement in BW and FCR with 1 or 2 g/kg inclusion.	ND	[140]
Thymol and carvacrol	200 or 300 mg/kg of feed	Improve BWG during challenge period ($P < 0.05$).	ND	[141]
Eugenol and garlic tincture	100 mg/kg of feed	Overall FCR was improved 1.681 PC vs 1.645 EOs	0.5 vs <0.5 (PC vs EOs—only in males).	[129]

*PC: Positive control; [€]ND: Not determined.

microbiome composition associated with EOs treatment is suggested to be a primary beneficial factor related to application of EOs as natural alternatives to antibiotics.

Combinations of EOs and organic acids have synergistic or additive effects that may improve poultry gut health and growth performance [137]. Similar to dietary EOs fed alone, blends of EOs and organic acids alter the composition of the gut microbiota, specifically increasing the abundance of Lactobacillus spp. [137] and SCFA concentration in the gut [142]. As a result, the dietary blends can inhibit the overgrowth of *C. perfringens* in the gut perhaps lowering the incidence and severity of NE. For instance, encapsulated blends of EOs (thymol, vanillin, eugenol) and organic acids (fumaric, sorbic, malic, citric) have been shown to improve gut health and performance of NE-affected broiler chickens

[143]. Similarly, feeding microencapsulated blends of EOs and organic acids (BUTYTEC-PLUS or ACITEC-MC) to broiler chickens placed on used NE litter increased growth performance due to improved intestinal barrier function and integrity [132]. Enteric inflammation associated with NE may have been reduced due to the anti-inflammatory effects of a dietary blend of encapsulated EOs and an organic acid (4% carvacrol, 4% thyme, 0.5% hexanoic, 3.5% benzoic, 0.5% butyric acid) [128]. Additionally, broiler chickens that received the EO and organic acid blend had improved intestinal integrity compared to the non-treated, challenged group [128].

Several investigators have demonstrated that EO supplementation has a positive effect on intestinal microbiota while also improving growth performance [8] [144] [145]. According to their findings, modulating broiler gut microbiota composition and activity with EO is an effective way to improve broiler performance.

Taken together, phytogenic compounds, such as EOs show promise as natural alternatives to mitigate the severity of NE-induced intestinal damage and performance losses. However, factors including the antimicrobial activity of the specific EO evaluated, EO dose, NE challenge model, and methods to determine efficacy of these naturally occurring AGP alternatives must be considered when designing experiments and comparing research findings.

2.5. Brief Overview of Methods to Evaluate Impact of Antibiotic Alternatives on Intestinal Integrity and Enteric Inflammation

Researchers have used different enteric inflammation models to understand the mechanism of action of multiple alternatives to antibiotic growth promoters (AGP) such as EO. Some of the models included nutritional factors [47], management [146], chemicals [147] [148], pathogen exposure [149] and environmental fluctuations [103] as challenge conditions to evaluate the effect of AGP alternatives on enteric inflammation. A non-terminal approach, such as serum fluorescein isothiocyanate-dextran (FITC-d) concentration, can be used to assess intestinal permeability and tends to correlate with bacterial translocation in the liver [150]. For the FITC-d assay, a 4 - 6 kDa FITC-d molecule is utilized since it cannot translocate through an undamaged intestinal epithelium [151]. Thus, an increase in FITC-d in the serum indicates that there has been damage to the intestinal epithelial barrier [151]. Other reliable serum biomarkers, such as antioxidant biomarkers, isoprostane 8-iso-PGF2 and prostaglandin GF2, have been evaluated [107]. Enterocyte biomarkers such as peptide YY, Fenterocellular signal-regulated kinase, citrulline, and mucin 2, as well as immune biomarkers peptide YY, enterocellular signal-regulated kinase, citrulline, and mucin 2, total or specific secretory IgA and interferon-gamma have been utilized [152] [153]. IgA is closely associated with mucosal immunity in mammals and avian species [154] [155]. It is the main immunoglobulin isotype in most mucosal secretions [155]. Therefore, elevated IgA levels can be associated with elevated mucin production and an increased immune response. Interferon-gamma is a pro-inflammatory cy-

tokine associated with intestinal inflammation and gut leakage [156]. Thus, interferon-gamma levels in the serum can be used to assess inflammation. Reactive oxygen species, such as superoxide are free radicals that are created naturally through cellular respiration. Free radical accumulation is damaging [157]. An enzyme, superoxide dismutase catalyzes superoxide into oxygen and hydrogen peroxide [158]. Superoxide dismustase concentration in the sera has been used to assess oxidative stress in broiler chickens [152]. Additionally, gene expression of other biomarkers, such as 1-acid glycoprotein, fatty acid-binding protein, and interleukins (IL-8, IL-1β,), mucin 2, transforming growth factor, and tumor necrosis factor have also yielded promising results [31] [159].

Inflammation alters expression of intestinal tight junction proteins followed by increased intestinal permeability [160]. Futhermore, intestinal morphometric measurements, such as villus height, villus width, crypt depth, and crypt/villi ratio can be used to evaluate gut integrity. An increase in crypt depth and villus witdh was indicative of gut barrier failure in broiler chickens [159]. The I See Inside (ISI) methodology, which employs both macroscopic and histological analyses, has been used to determine the impact of a treatment or challenge on an organs function. This method been used to assess effect of EOs and organic acids on ISI scores in NE-challenged broiler chickens [143].

3. Conclusion

The removal of AGPs or shift to antibiotic-free or no antibiotics ever production in commercial poultry systems has been associated with reduced performance and increased mortality [161] [162] [163]. Diseases that were traditionally treated by subtherapeutic amounts of antibiotics in the diet have increased. This has had a negative effect on the health of commercial chickens and has incurred substantial costs for poultry producers. To counteract this, the industry has begun to explore alternatives to antibiotics for treating impending health problems, such as NE. Even though CP is a commensal in the avian intestinal tract, dysbiosis produced by inflammation and compromised intestinal integrity encourages the uncontrolled proliferation of CP. Infectious pathogens, such as *Eimeria* maxima, appear to be the most important risk factor for NE. However, any kind of chronic stress, regardless of its origin (nutritional, environmental, physical, chemical, or psychological) that alter the microbiota-brain-gut axis are also linked to NE. Due to their antibacterial, antiviral, antifungal, anti-inflammatory, immunomodulatory, epithelial barrier, microbiota modification, and antihyperlipidemic characteristics, EOs have become more popular as feed additives over the past two decades. Moreover, there are an outstanding number of studies suggesting that EOs are a safe and effective alternative to antibiotics to reduce the incidence and the severity of NE in broiler chickens. In conclusion, EOs can be used in poultry feed, but there are still questions about their action, metabolic pathway, and optimal dosage in poultry that need to be investigated further.

Author Contributions

MEC, BDG, and GT-I developed the conceptualization and wrote the first draft of the manuscript. GT-I drew and edited the figures. VP-G, XH-V, XS, JDL, and BMH participated in design, analysis, presentation, and writing of manuscript. All authors have read and agreed to the submitted version of the manuscript.

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All figures were created with BioRender.com. Macroscopic photographs of organs with lesions, clinical signs or mortality were taken by VP-G and XH-V. Microscopic photographs were taken by BDG.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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