An Overview of Advanced Use of Essential Oils for Poultry Health and Disease Management

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Abstract
In this review, we discuss how essential oils (EOs) can be used in poultry farming as a sustainable alternative to conventional practices. The study brings to focus the industry's most urgent issues: antibiotic resistance and improved health, pushing for alternative solutions. Given their antimicrobial, antioxidant and growth promoting capability, EOs are highlighted as potential substitutes with their rich composition of terpenes and phenolics. The review approach how EOs promote poultry health with regard to growth promotion, improving immune function and enhancing feed conversion ratios. It further examines their impact on gut health, disease resistance, and the potential to elevate product quality. The integration of EOs into poultry practices is presented as a strategy to mitigate environmental challenges. We aimed to provide a review of the biological mechanisms behind the efficacy of EOs in managing diseases and promoting health in poultry, against a backdrop of various pathogens.

Keywords: antibiotic alternatives, biological mechanisms, disease management, essential oils, poultry production

1. Introduction

Currently, with chickens on the path to becoming the most consumed meat worldwide and considering their potential role as reservoirs for zoonotic diseases, proper farm management has become a critical priority within the One Health framework, which emphasizes the interconnectedness of animal, human, and environmental health [1]. Concomitant with the increase of meat production, the escalating persistent problem of bacteria becoming resistant to antibiotics is linked to the excessive and often unnecessary application of these drugs in both veterinary alternatives to antibiotics, represented by different plant extracts, EOs, organic acids and natural antimicrobial mixtures [2, 3].

EOs have been identified as utile components in the development of novel strategies for disease management, presenting an innovative and interesting approach to biosecurity in poultry farming. Their ability to act against various pathogens (i.e. *Salmonella* and *Campylobacter* spp, including *E. coli* and others) positions them as important in maintaining poultry health and welfare [2-5]. Moreover, the Food and Drug Administration (FDA) in conjunction with the Flavor and Extracts Manufacturers Association (FEMA), have acknowledged the safety of EOs for food industry applications, incorporating them into the Generally Recognized as Safe (GRAS) indexes [6]. Integrating natural products such as EOs into agricultural practices has not just become a trend but is also a paradigm change in sustainable farming, particularly within the poultry industry.

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As antibiotic resistance threatens as a global threat and environmental sustainability becomes a mandatory rather than a choice, EOs have emerged as promising candidates to address these issues with their multifunctional properties.

They have been used as feed additives to improve performance for all species including many avian species, ruminants and monogastric, especially because of their antimicrobial properties, but also to modify microbiota [7, 8]. They can interact with microbial cell membranes and inhibit the growth of some gram-positive and gram-negative bacteria [7, 9]. These natural extracts are supported with multiple bioactive compounds such as terpenes, phenolics, alcohols, ketones, aldehydes and other important compounds, which are reported to exhibit potent antimicrobial, antifungal, and antioxidant activities [10-12].

Regarding disease management, EOs have recently demonstrated various anti-infectivity modes of action in poultry against enteric diseases induced by *Eimeria*, *Salmonella* and *Clostridium* spp [13-15]. Different concentrations (e.g. 0.25 ml/L or ranging from 200-500 mg/kg) of peppermint, eucalyptus, carvacrol, cinnamaldehyde and thyme EOs added in the poultry diets have been demonstrated to ameliorate the gut injuries, increase mortality and resilience versus these pathogens [13-15]. Moreover, several studies have suggested that EOs could exhibit a higher biological efficacy, especially antimicrobial activity when are combined with organic acids, due to synergistic or additive indirect properties [13-16]. One possible explanation is attributed to the pronounced hydrophobic nature of EOs that enhances their ability to permeate bacterial membranes, potentially increasing membrane permeability and thus aiding the entry of organic acids into the bacterial cytoplasm [16].

The present review is dedicated to delivering an assessment of the potential roles of EOs within poultry, scrutinizing their effects and exploring the feasibility of incorporating these natural entities into the poultry industry. By analyzing contemporary research and outcomes, the paper seeks to furnish detailed perspectives on how EOs could elevate poultry production through sustainable practices, focusing on the biological mechanisms and effectiveness of EOs in disease management against various pathogens.

2. Biological mechanisms of action of essential oils linked to poultry

EOs have accumulated significant attention as poultry feed additives in recent years due to their multifaceted biological properties. These include antimicrobial actions against bacteria, viruses, and fungi and anti-inflammatory and immunomodulatory effects [11, 17]. EOs also enhance the integrity of epithelial barriers, modulate gut microbiota, improve animal production performance, and exhibit anti-hyperlipidemic traits [17]. These natural products been demonstrated to encourage the production of intestinal mucus in broiler chickens [12]. This increase in mucus can obstruct the adhesion and invasion of pathogenic bacteria to the gut lining, thereby reducing infection risks. Additionally, this action contributes to maintaining a balanced state of gut microbiota, known as eubiosis, which is important for the overall health and productivity of poultry [12]. These aromatic compounds are known to play a significant role in augmenting the production of digestive enzymes, thus promoting more effective digestion and nutrient assimilation [18]. They modify the palatability of feed by improving its flavour and aroma while also facilitating protein digestion through the elevation of gastric secretions and hydrochloric acid levels [18]. However, the selection for supplementation must be done with caution, as certain types can irritate the epithelial lining of the intestines and potentially lead to inflammatory responses. Therefore, it is noteworthy to identify and use those EOs that offer beneficial effects without adverse impacts on the intestinal health of the animals.

The mechanisms underlying the effects of EOs have been the subject of in-depth reviews. They are known to inhibit the growth of both Gram-negative and Gram-positive bacteria *in vitro*, primarily by augmenting the permeability of the bacterial cell wall and mitochondrial membranes [11]. The antimicrobial potency of EOs is notably influenced by the hydrophobicity of their compounds, which facilitates their interaction with lipid bilayers. The proposed antibacterial mechanism of action for EOS involves the disruption of the bacterial cytomembrane [11].

This breakdown compromises the integrity of the bacterial cell, leading to modification of bacterial pH and the leakage of vital intracellular components such as proteins and potassium ions (K^+), which is detrimental to bacterial survival [11, 12]. Furthermore, EOs provide a unique substrate that support the growth and proliferation of beneficial intestinal lactic acid bacteria (LAB), such as probiotics species, namely *Lactobacillus* species, which can enhance gut health and outcompete pathogenic bacteria [12]. To combat salmonellosis, early research suggests that eugenol exerts its antibacterial effects primarily by disrupting the cell membrane of *Salmonella* [19]. This disruption impedes ATP synthesis, effectively starving the bacterial cells of energy and resulting in cell death. In a controlled *in vitro* model using chicken cecum, the application of eugenol was shown to reduce *S. Enteritidis* populations by over five log10 CFU/mL, indicating a potent antibacterial activity [19].

Beyond their antimicrobial activity, EOs are particularly lauded for their antioxidant and anti-inflammatory capacity, specifically under different stress conditions. These factors are significant in preventing lipid peroxidation within cell and mitochondrial membranes and safeguarding proteins and DNA from denaturation [17]. Consequently, EOs play a pivotal role in averting diseases and organ failure by protecting cellular components from oxidative damage. These attributes contribute to enhanced poultry performance by mitigating oxidative stress and reducing inflammation, which can otherwise compromise health and growth [10].

Star anise and thyme EOs contain phenolic OH groups within their molecular structures, which act as hydrogen donors [20]. This ability allows them to neutralize peroxyl radicals, which are primary agents in initiating lipid oxidation, thereby impeding the formation of lipid hydroperoxides. In practical applications, supplementing poultry diets with star anise EO has been shown to elevate TAC in both laying hens and broilers, indicative of a strengthened systemic defence against oxidative stress [20]. Star anise oil at a dosage of 494 mg per kilogram of feed or an equivalent concentration in drinking water, there is consensus that it poses no risk to chickens raised for meat, as well as other poultry species reared for similar purposes [21]. Further extending these safety considerations, the European Food Safety Authority (EFSA) has also endorsed using this additive at 1.9 mg per kilogram of feed for egg-laying hens [21]. Notably, this recommendation also applies to a broader spectrum of avian species, including those bred for reproductive and ornamental reasons, as well as game birds. This extrapolation derives from the assumption that the physiological responses observed in laying hens would reflect those in other related bird categories.

The endogenous antioxidants in poultry and other animals, namely superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and overall total antioxidant capacity (TAC), proved to play an important role as superoxide scavengers and anti-inflammatory agents to fight against different diseases [20, 22, 23]. Two EOs (*Plectranthus amboinicus* and rosemary) were recently evidenced to show *in vivo* antioxidant capacity in broilers at 100 mg/kg [22]. The authors reported significant boosts of antioxidant plasma markers such as GPx, SOD, and TAC, as well as decreases of MDA from broiler breasts and thighs after nutritional supplementation. Specifically looking at thyme EO, its impact on critical antioxidant enzymes has been notable [20]. Thyme EO administration has been associated with a significant increase in the hepatic activities of CAT, SOD, and gGSH-Px [24]. These enzymes protects the tissues from oxidative damage; CAT and SOD are involved in detoxifying harmful oxygen radicals, while GSH-Px also plays a crucial role in protecting intracellular lipids from peroxidation [20]. Again, the inclusion of clove, peppermint, and cinnamon oils separately at 0.3 % concentration or a combined level of 0.1% in the diets of broiler chicks significantly enhanced serum total protein levels, GPx activity, and high-density lipoprotein (HDL) concentrations [25]. Conversely, this dietary strategy notably reduced serum COL, TG, low-density lipoprotein (LDL), and lipid peroxidation (as measured by MDA) concentrations. The research concludes that using a blend of these oils in the diet can positively impact performance, blood biochemistry, and economic efficiency without adverse health effects under the conditions tested [25].

For instance, phenolic compounds present in EOs have been observed to elevate the activity of CAT, an enzyme that plays a essential role in detoxifying hydrogen peroxide by breaking it down into water and oxygen [26]. Additionally, they assist in converting lipid hydroperoxides, which can be harmful, into non-toxic substances, thereby protecting cells from oxidative damage. In addition, diets enriched with EOs, such as from *Lippia origanoides* may enhance the growth of broiler chickens, particularly under conditions of cyclic heat stress [10]. This finding is particularly pertinent for antibiotic-free poultry production systems that operate in regions with extreme climate conditions.

In an recent investigation of the impact of heat stress on poultry, parameters including feed intake, weight gain, and feed conversion were monitored alongside the upregulation of the heat-shock protein (HSP70) in the liver and jejunum [27]. Heat stress was observed to decrease feed intake and WG while increasing the feed conversion ratio, indicating a decline in performance. When a blend of EOs containing carvacrol, cinnamaldehyde, and eugenol was included in the diet at a 3% concentration, there was an upregulation of HSP70 gene expression in the jejunum and liver of broilers at 42 days of age. This insight suggests that at this inclusion level, the EOs may induce a stress response. On the other hand, a lower inclusion level of 1.5% of the EOs or the control diet without EOs led to lower HSP70 expression in both tissues. This could imply that at 1.5% inclusion or none, the broilers could better manage heat stress, as indicated by the reduced. need to express HSP70, a protein typically associated with stress response and cellular protection [27]. Another related finding suggested that heat stress triggered an upregulation in broiler chickens of genes associated with antioxidant activity, apoptosis, detoxification, and heat shock responses, with the exception of Bcl-2, which is often involved in preventing apoptosis [28]. Cumin EO, when administered, exerted a dose-dependent beneficial effect on certain genes within the tissues of the heat-stressed broilers. It appeared to downregulate the same set of genes that were upregulated due to heat stress, again with the exception of Bcl-2 [28]. This indicates that cumin EO may have a protective role against the cellular stress responses induced by heat stress in broilers [29]. Different comparative analysis revealed that cumin EO administration to heat-stressed broiler chickens resulted in a notable amelioration of lipid peroxidation both in plasma and tissue levels when contrasted with the control group, which indicates a reduction in oxidative stress [29]. Furthermore, there was a decrease in the activity of antioxidant enzymes, suggesting that less reactive oxygen species were present due to the protective effect of the cumin EO. Again, supplementation was also found to have a beneficial impact on serum biochemistry, indicating improved physiological health, leading to an increase in the populations of beneficial microorganisms within the small intestine, which indicates a positive modulation of the gut microbiome. This enhancement of beneficial intestinal flora can contribute to better nutrient absorption and a stronger gut barrier against pathogens. Collectively, these findings suggest that cumin, carvacrol, cinnamaldehyde, and eugenol EOs not only mitigated the negative impacts of heat stress on broiler chickens but also contributed to improvements in their health and productive performance, showcasing its potential as a valuable dietary supplement in poultry management under conditions of thermal stress.

3. Essential oils in disease management

Globally, the poultry industry incurs economic losses exceeding $3 billion due to avian coccidiosis [30]. The efficacy of EOs against a broad spectrum of pathogens not only opens new avenues for disease management but also enhances overall poultry health and productivity. To elucidate the mechanisms by which EOs exert their beneficial effects in poultry, Figure 1 provides a schematic representation, illustrating their modes of action, including antimicrobial activity, stimulation of the immune response, and improvement of gut health.

The mucosal damage caused by coccidiosis can predispose poultry to secondary bacterial infections leading to necrotic enteritis (NE), with common pathogens including *Salmonella enterica* Typhimurium, *Clostridium perfringens and E. coli*. Exposure to parasites such as *Eimeria* at an early age in broilers may damage intestinal integrity, compromise their survival rates, serum antioxidant capacity later in life and adversely affect the quality of the meat [30, 31]. *In vitro* studies effectuated on primary chicken epithelial cells have recently indicated that oregano and thyme EOs, including carvacrol and thymol, significantly reduced the levels of interleukin expression in Eimeria-infected cells [32]. The authors stated that the most impactful effects against *E. tenella* sporozoites morphology and invasion were noticed in carvacrol and thymol after ≈2h of postinfection.

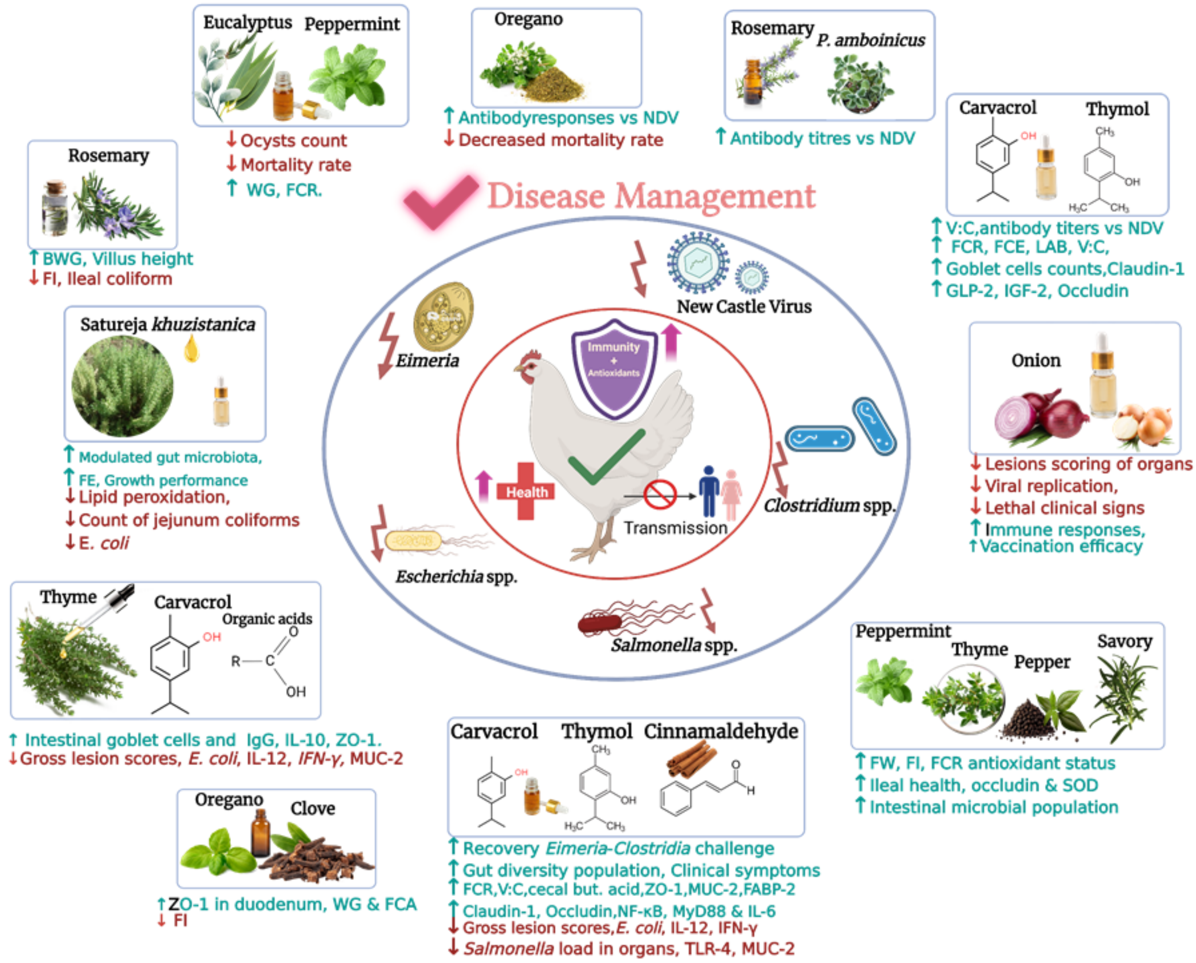

Figure 1. The latest updates of different EOs and their solely dietary application or in combination for disease management in poultry against bacterial, viral and parasitic pathogens. Symbol ↑ - represents increases or enhancement of investigated parameter. Symbol ↓ - represents a reduction, decrease or downregulation of reported measured variable. Created with *Biorender.com*

Furthermore, EOs limited the pro-inflammatory cytokine production related to *Eimeria*, *C. perfringens*, *E. coli*, and *Salmonella* spp. challenge [15, 17, 33, 34]. Other studies have investigated the therapeutic effects of additives (4% carvacrol and thyme) mixed with organic acids on broiler chickens infected with NE triggered by *Eimeria* spp./*C. perfringens* [15]. Adding blend at 200 mg/kg and 500 mg/kg dosages improved gut health significantly, evidenced by reduced gut damage, lower levels of harmful bacteria in the liver, and better gut morphology. Furthermore, the treatment altered the gut's gene expression beneficially and improved the gut microbial composition, enhancing growth performance comparable to antibiotic growth promoters (AGPs). Their findings suggested that the mixture containing EOs and organic acids could be a potent alternative to AGPs in managing gut health in NE-infected broilers. Incorporating clove or oregano EOs into the diet of broilers at a concentration of 500 ppm has been found to enhance their overall performance and can mitigate some of the damage to gut integrity and meat quality caused by coccidial *Eimeria* infection [31]. A recent study has concluded that a blend of EOs based on eucalyptus and peppermint EOs at 0.25ml/L can be used as an alternative treatment to prevent coccidiosis caused by *Eimeria* in the poultry industry [13]. The authors proposed that a synergistic blend of EOs alongside conventional disinfectants may protect broiler chickens against the growth and immune disruptions caused by coccidiosis.

For example, the pathogenicity of *C. perfringens* was modulated by the inclusion of a mixture of EOs from 25% carvacrol and 25% thymol at 120 mg/kg in the diet which downregulated *in vivo* expression of *C. perfringens* virulence factors [35]. Furthermore on a par results have also been illustrated in the earlier research of Du et al., 2016 [36]. Similarly, new studies have shown an EO complex mainly containing 5% of 1:1 thymol and carvacrol, which was dietary administered at 1,000 -2,000 mg/kg to *C. perfringens* and coccidia-challenged layer hens that assisted in the recovery of birds from the challenge by enhancing production performance, improving the diversity of gut microbial populations and supporting the overall intestinal health compared to the control group [34, 37]. Interestingly, different oil cakes fabricated from EOs plants at 1-4% concentration did not exhibit any antimicrobial capacity; however alleviated the levels of *E. coli*, *Enterococcus*, and *Proteus* in the artificial intestinal chicken model [38]. The authors stated that the reducing capacity was attributed to the stimulation of intestinal *Lactobacillus* populations that produced lactic acid and lowered the pH of the medium.

Besides coccidiosis, avian colibacillosis, primarily induced by avian pathogenic *E. coli* (APEC), is a prominent bacterial affliction within the poultry sector. Chronic infections of a mild to moderate nature attributable to APEC are associated with a spectrum of adverse outcomes, including diminished feed consumption, reduced live weight, lower egg production and hatchability, suboptimal FCR, and escalated rates of carcass contamination during processing, which in turn pose a potential threat to human health [33]. Previous *in vitro* studies have evidenced that carvacrol and thymol can inhibit biofilm growth and formation of avian pathogens such as *E. coli O78* and *Salmonella pullorum* by 95% [39]. In an exploration of EOs additives as dietary interventions against *E. coli* infections in poultry, it was found that EOs (4% carvacrol and thyme) combined with organic acids 500 mg/kg feed supplementation resulted in improved FCR and lessened intestinal lesion severity [33]. Immunologically, EOs prompted a decline in *E. coli* populations in the cecum, maintained intestinal goblet cell prevalence, increased serum IgG, and tended to reduce IL-12 serum levels. Gene expression analysis revealed a downregulation of pro-inflammatory IFN-γ mRNA and an upregulation of the anti-inflammatory IL-10 mRNA, indicating a shift towards a less inflamed gut environment. The 16S rRNA gene sequencing highlighted that the supplementation induced notable changes in the cecal microbiota's diversity and composition, particularly reducing Bacteroidetes in infected birds. Such research suggests the combinations of EOs and organic acids as a promising feed additive to ameliorate pathogen-induced gut inflammation and support the intestinal health of poultry, presenting a potential alternative to traditional antibiotics in poultry management [33]. Other research found that dietary inclusion of *Satureja khuzistanica* EO enhances broiler performance and positively modulates gut microbiota [40]. Notably, microencapsulating the oil with alginate bolstered its efficacy, leading to superior growth performance, improved FE, and heightened antibacterial action, which culminated in a reduction of pathogenic gut bacteria, specifically *E. coli*. The study recommends the administration of 100 mg/kg of this microencapsulated EOs in broiler diets as it yields improvements in bird performance, meat oxidative stability, and gut microbial balance [40]. In another *in vivo* experiment, the administration of both non-encapsulated and encapsulated EOs from *R. officinalis* at varying dosages (150 or 300 mg/kg) significantly enhanced the body weight (BW) gain and feed conversion ratios of the broilers when compared to those on control and antibiotic regimens [41]. Notably, the ADFI was reduced in groups supplemented with higher concentrations of EOs (150 or 300 mg/kg). Moreover, adding EOs to the diet resulted in a marked increase in villus height within the birds, suggesting improved nutrient absorption. Additionally, a lower ileal coliform count was observed in birds receiving 150 mg/kg of non-encapsulated EOs, indicating a potential antibacterial effect when compared to the control diet. The *in vitro* results confirmed a significant antimicrobial effect of encapsulated EO against *E. coli* and *Staphylococcus aureus* with an estimated IC_{50} values of 0.969 and 1.879 mg/mL, respectively [41]. Prior research has illustrated the efficacy of EOs in combating multidrug resistance (MDR) in *Staphylococcus epidermidis* by targeting key resistance mechanisms: (1) suppressing efflux pump activity, (2) hindering Extended Spectrum Beta-Lactamases (ESBLs) such as plasmid-mediated AmpC, TEM-1, TEM-2, and SHV-1 in Gram-negative bacteria, and (3) obstructing resistance genes like mecA, mecR1, mecI, blaZ, blaR1, and blaI [1].

Salmonella spp. stands as a principal cause of foodborne diseases globally, presenting a significant public health concern. *Salmonella* contamination originating from poultry is estimated to inflict financial damages amounting to approximately $2.8 billion in the United States, highlighting its substantial economic impact among various foodborne pathogens [19]. These bacteria, across various serovars, are known to infect a wide range of domestic animals such as cattle, poultry, pigs, and sheep [19]. The clinical presentation in these animals can vary from mild gastroenteritis to fatal outcomes in severe instances of infection. For example, a study concluded that the fabricated microcapsules containing a blend of EOs—namely thyme (50%), peppermint (12.5%), savory (25%), and black pepper (12.5%) exhibited antioxidant and antibacterial properties [42]. However, when used as a dietary supplement in the feed of broiler chickens at concentrations of 0.5, 1, and 2 kg/ton, they effectively improved various parameters in chickens challenged with *S. Enteritidis*. These parameters included the final BW, total feed intake, feed conversion ratio (FCR), and overall antioxidant status of the body. Additionally, positive effects were observed on the ileal morphostructure, intestinal microbial balance, and the expression of genes related to antioxidant activity and inflammation in the ileal tissue [42]. Last investigations found that adding a mixture of coated EOs (with concentrations of thymol 8.0%, carvacrol 8.0% and cinnamaldehyde 5%) and organic acids to the broiler diet at dosages of 500 mg/kg and 800 mg/kg can mitigate the detrimental impacts of *Salmonella* enterica infection [14]. This mitigation is evidenced by improved intestinal structure, diminished *Salmonella* Enteritidis counts in the liver, spleen, and cecum, and an increased mRNA expression of key tight-junction proteins [14]. Hens challenged with *S. Gallinarum* for the induction of fowl typhoid and treated with cinnamaldehyde at a 1:8000 dilution in drinking water exhibited notable improvements [43]. The benefits included enhanced egg production and milder clinical symptoms than the control group. Furthermore, these hens demonstrated a more efficient FCR and a significantly reduced bacterial presence in the ceca and on the eggshell surface [43]. Other studies demonstrated that thyme EOs applied in poultry litter at 1.25%-5% concentration revealed a potent antimicrobial activity by hindering *E. coli* and *Salmonella* Derby between 73.3% and 77.8%, respectively [1]. The authors conclude by endorsing the potential use of this natural method for treating poultry litter, presenting it as an effective strategy to prevent diseases caused by prevalent bacteria in poultry farms, thereby contributing to the effort against the emergence of antibiotic resistance.

Finally, the oral delivery of *Allium cepa* EOs alongside Newcastle disease virus (NDV) vaccines notably diminished and eradicated severe clinical symptoms and lesions associated with the velogenic NDV [44]. Furthermore, the authors reported that *A. cepa* EOs with the vaccine curtailed viral shedding, amplified the immune response, and increased the protection rate post-challenge. Two EOs from *P. amboinicus* and rosemary at 100 mg/kg showed antiviral activity in broilers by enhancing their antibody titres and blood parameters against NDV [22]. Incorporating oregano EO into the drinking water of broiler chickens at a concentration of 100 mg/L has yielded positive outcomes on their growth and immune function [45] leading to increased BW, an enhanced profile of white blood cells, and improved phagocytic immune responses, indicative of an overall boost in the birds' natural defence mechanisms. Moreover, oregano oil has shown a remarkable capacity to counteract the adverse effects of the immunosuppressive agent cyclophosphamide. It not only mitigated the reduction in BW and deterioration of immune responses caused by cyclophosphamide but also elevated the antibody responses against the NDV and stimulated the proliferation of lymphoid organs. These benefits were also associated with a decreased mortality rate, underscoring the potential of oregano as a supportive agent for immune health and survival in poultry.

4. Conclusions

EOs have exhibited promising results in disease management in the disease management division by reducing diseases caused by avian pathogens that place a heavy economic burden on the poultry industry. Due to the reversible modulation of immune responses and enhancement of tissue integrity, their usage can be a sustainable alternative to combat poultry diseases without inducing resistance. EOs efficacy and safety have to be evaluated contextually for comparative studies due to variations in response based on differences in their composition, dosages, and bird breeds.

Finally, strategic integration of EOs by the poultry farming industry is not only an adaptation to consumers preference and regulatory changes but also an endorsement of a wide range approach to farm animals rearing. They are at the intersection of tradition and innovation and the only viable and sustainable way to meet the soaring demands of the global poultry market. Encouragingly, as we move down the road, it will be critical to continue to adapt the EOs along with probiotic and prebiotic supplementation strategies in such a way as to maximize both bird performance and welfare to create a strong and productive poultry sector.

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