ESSENTIAL OILS AND THEIR EFFECT ON GROWTH PERFORMANCE, INTESTINAL HISTOMORPHOLOGY, BLOOD, CARCASS AND IMMUNE ORGAN CHARACTERISTICS OF BROILER CHICKENS: A COMPREHENSIVE REVIEW

OGBUEWU, I. P.* – MABELEBELE, M. – MBAJIORGU, C. A.

Department of Agriculture and Animal Health, University of South Africa, Florida Science Campus, Private Bag X6, Florida, 1710, South Africa

**Corresponding author*
e-mail: dr.ogbuewu@gmail.com, ifeanyi.ogbuewu@futo.edu.ng;
ORCID: 0000-0003-4895-7867

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Abstract. The use of eco-friendly feed additives, such as plant essential oils (EO) as performance-enhancing agents has gained attention in animal production. Studies suggest that EO, with bioactive components such as phenols, lectins, polyphenols, and terpenoids, can improve gut health, immune response, product quality, growth dynamics, and nutrient utilization in chicken diet. These improvements may be partly attributed to the antimicrobial, immunostimulatory, and antioxidant effects of EO in avian species. In addition, EO reduces blood and meat cholesterol levels, as well as abdominal fat in birds, by hindering the activities of several enzymes linked to cholesterol and fat formation. However, some authors found that dietary EO supplementation does not affect broiler chicken performance. These variable results in broiler chickens fed EO-supplemented diets make it challenging to use this large volume of information in evidence-based decision-making. Thus, this review was designed to ascertain the effectiveness of dietary EO supplementation in improving growth performance, intestinal histomorphology, blood, carcass, and immune organ status of broiler chickens to increase the uptake of these findings in the poultry industry. The bioactive components, biological activities, and mechanisms by which EO elicits their positive effects on broiler chicken performance are also discussed, along with limitations to their use in broiler chicken production.
Keywords: *phytogenics, bioactive compounds, biological activity, poultry, weight gain, health markers*

Introduction

Global broiler chicken meat production reached 103.5 million metric tons in 2023 and is projected to increase to 104.2 million metric tons by 2025 (Shahbandeh, 2024). To achieve this target, there is a need to optimize chicken performance and health using specific feed additives. Phytogenic feed additives, which include plant-based substances such as extracts, spices, herbs, and essential oils, contain bioactive and flavoring compounds. Essential oils, also called volatile oils, are biologically active organic substances obtained from different plant parts (seeds, bark, stems, roots, etc). Essential oils are not truly lipids and are extracted via steam distillation or solvent extraction methods. The composition and concentration of EO in a single plant can vary depending on the specific part used (Puvaca et al., 2022). Studies have shown that EOs are responsible for the aroma of plants and spices and they often have a wide variety of antimicrobial activities (Chao et al., 2000; Mangalagiri et al., 2021). Over 3000 EO have been identified, with 10% considered to be of commercial or economic significance (Nehme et al., 2021). Essential oils are deemed safe as feed additives in animal nutrition (FDA, 2004) and on this basis, the use of EO in animal production is on the increase owing to their capacity to promote gut health (Irawan et al., 2021;

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Caroprese et al., 2023; Islam et al., 2024). Thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum verum*), oregano (*Origanum vulgare*), and rosemary (*Rosmarinus officinalis*) oils are the common EO used in the poultry industry to improve broiler chicken performance and health. However, there are inconsistent findings on the impact of dietary EO on growth dynamics, carcass characteristics and health status of broiler chickens (Yang et al., 2019; Irawan et al., 2021; Elbaz et al., 2022; Caroprese et al., 2023; Ghazanfari et al., 2024; Huang et al., 2024; Islam et al., 2024), thus making it difficult to use these data in decision-making process. Presently, there is little or no published review on the impact of EO additives on growth dynamics and health status of broiler chickens in the literature. The use of review as a tool by researchers to understand the current state of knowledge on a particular topic has been highlighted (Zeng et al., 2020; Puvaca et al., 2022). Thus, the objective of this study was to pool published studies on the effect of EO supplementation on broiler productivity and health to enhance the uptake of these findings in the poultry industry. The bioactive components, biological activities, and mechanisms by which EO elicits their positive effects on broiler chicken performance are also discussed, along with limitations to their use of EO in broiler chicken production.

Methodology

The articles used for the study were retrieved via a search done on Google Scholar, DOAJ, ScienceDirect, PubMed, JSTOR, Web of Science, AGORA, BioMed Central, and Scopus. The search was done using the following search keywords and phrases: broiler chickens, essential oils, pharmacological effects, antioxidants, antimicrobial, growth performance, blood characteristics, hematology, serum biochemistry, internal organs, carcass yield, cut-part, meat quality, intestinal histomorphology, and immune responses. The search queries used to retrieve articles from the various databases were Boolean operators, truncation, and wildcards.

Essential oils and their bioactive components

Essential oils are concentrated blends of many volatile substances, which most often are colorless or yellowish. They are also called secondary metabolites since they are produced from primary metabolites during plant metabolism (Dehariya et al., 2020). They are highly soluble in organic solvents, like gasoline, ether, chloroform, and others. Most EO has a viscosity comparable to water or alcohol, while others are sticky and viscous. Essential oils are smaller molecules and as a result are not considered as lipids (Ríos et al., 2016). The boiling points of EO are hard to determine due to their heterogeneous composition. However, their boiling temperature ranges from 150°C to 280°C, allowing the separation of individual components by fractional distillation (Turek and Stintzing, 2013). Other techniques for extracting EO from plants include hydrodistillation (Lucchesi et al., 2004), extraction with organic and non-volatile solvents (Ferreira et al., 2020), cold pressing (Bento et al., 2020), and extraction with solvents aided by microwave action (Filly et al., 2014). The extraction method to be used is determined by the nature and type of plant, as well as the economic viability of the technique and the intended use of the oil. The amount of EO produced from a single plant varies from 0.2 to 2.0%, except for rose EO, which yields no more than 0.03%, and clove EO, which yields up to 2.0% (Lukas et al., 2015).

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Essential oils most often contain tannins, terpenoids, flavonoids, polyphenols, polypeptides, and a variety of other substances that make individual EO distinct (Wink, 2015). Bioactive substances in EO can be categorized into two main groups, including terpenes (monoterpenes and sesquiterpenes) and oxygenated compounds. Terpenes are made from isoprene units, which are blends of two isoprene units, termed terpene units. The oxygenated compounds in EO are synthesized from terpenes, often called "terpenoids." They include phenols, alcohols, aldehydes, ketones, esters, oxides, and several others (Tongnuanchan and Benjakul, 2014).

Tables 1 and *2* present the quantitative value of EO in selected plant species (Ainane et al., 2019; Abd El-Hack et al., 2020; Puvaca et al., 2022). The major bioactive compounds in thyme and oregano oils are thymol, cymene, and carvacrol (*Table 3*). Linalool (4.05%), α-Pinene (12.50%), 1,8-cineole (16.10%), and camphor (17.70%) were the predominant bioactive components in rosemary EO. The dominant group of chemical compounds in cinnamon oils are linalool and β-Caryophyllene, which agrees with the finding of Abdelwahab et al. (2017). The bioactive components of EO and oil yield differ among plant species, as illustrated in *Tables 1* and *2*. This variation could be attributed to factors such as plant age, plant part, genetically determined properties, and the extraction method used.

Table 1. *Effects of EO intervention on growth dynamics of broiler chickens*

References	Dosage (mg/kg)	EOs or their components	Species	Treatment effect*		
				BWG	ADFI	FCR
Saleh et al. (2014)	100	Thyme	Thymus vulgaris	+ 21	+ 27	+ 13
Saleh et al. (2014)	300	Thyme	Thymus vulgaris	+ 8	+ 0.3	–7.2
Zeng et al. (2015)	75, 150	Ginger	Zingiber officinale	+ 7	+ 6	0
Zeng et al. (2015)	250	Oregano	Origanum vulgare	+ 3	+ 4	0
Zeng et al. (2015)	1000	Thyme	Thymus vulgaris	–4	–3	0
Galal et al. (2016)	0.005^{++}	Oregano	Origanum vulgare	+ 2	–0.1	–3
Galal et al. (2016)	0.01^{++}	Oregano	Origanum vulgare	+ 2	+ 2	0
Yang et al. (2019)	50	Cinnamon	Cinnamomum verum	+ 1.3	+ 2	–0.01
Yang et al. (2019)	100	Cinnamon	Cinnamomum verum	–0.7	–1.1	–0.01
Yang et al. (2019)	200	Cinnamon	Cinnamomum verum	–0.03	–0.6	–0.01
Yang et al. (2019)	400	Cinnamon	Cinnamomum verum	+ 0.2	+ 0.2	0
Yang et al. (2019)	800	Cinnamon	Cinnamomum verum	+ 0.9	+ 0.8	0
Elbaz et al. (2022)	200	Garlic	Allium sativum	+ 2	+ 0.4	–0.1
Elbaz et al. (2022)	200	Lemon	Citrus limon	+ 4	+ 2	–0.04
Huang et al. (2024)	200	Thymol & carvacrol	Thymus vulgaris	+ 2	+ 0.2	–2
Huang et al. (2024)	400	Thymol & carvacrol	Thymus vulgaris	+ 0.4	–0.5	–1
Huang et al. (2024)	600	Thymol & carvacrol	Thymus vulgaris	+ 1	–2	–3
Huang et al. (2024)	800	Thymol & carvacrol	Thymus vulgaris	+ 0.3	–1	–1
Ghazanfari et al. (2024)	150	Peppermint	Mentha piperita	–13	–2	+ 0.4
Islam et al. (2024)	150	Thymol & carvacrol	Thymus vulgaris	+ 5	0	–5

* = Percentage difference from control group; $^{++}$ = ml/L; EO essential oils; BWG body weight gain; ADFI average daily feed intake; FCR feed conversion ratio

Biological activity of EOs

Research has shown that EO possesses numerous pharmacological effects such as antimicrobial, antioxidative, anti-inflammatory, antidiarrheal, and immunostimulatory

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(Gopi et al., 2014; Krishan and Narang, 2014; Abd El-Hack et al., 2020). The pharmacological effect of the EO is tied to their chemical composition, functional groups, and synergistic interactions that occur between their individual components (Tongnuanchan and Benjakul, 2014).

Table 2. *Effects of EO intervention on blood and meat cholesterol levels of broiler chickens*

References	Dosage (mg/kg)	EOs or their components	Species	Treatment effect*		
				TC	HDL-C	LDL-C
Ciftci et al. (2010)	500	Cinnamon	Cinnamomum verum	–21	-	–
Ciftci et al. (2010)	1000	Cinnamon	Cinnamomum verum	–15	-	-
[+]Ciftci et al. (2010)	500	Cinnamon	Cinnamomum verum	–16	-	–
[+]Ciftci et al. (2010)	1000	Cinnamon	Cinnamomum verum	–11	-	-
Saleh et al. (2014)	100	Thyme	Thymus vulgaris	–7	+ 5	–3
Saleh et al. (2014)	200	Thyme	Thymus vulgaris	–1	–7	+ 6
Saleh et al. (2014)	300	Thyme	Thymus vulgaris	+ 17	–4	+ 20
Saleh et al. (2014)	100	Ginger	Zingiber officinale	–3	–2	+ 78
Saleh et al. (2014)	200	Ginger	Zingiber officinale	–5	–11	+ 10
Saleh et al. (2014)	300	Ginger	Zingiber officinale	–7	+ 1	+ 6
Pathak et al. (2016)	500	Cinnamaldehyde	Cinnamomum verum	–8	–9	–16
Iqbal et al. (2021)	100	Nr		+ 26	+ 8	–20

* = Percentage difference from untreated control; [+] = breast meat cholesterol; nr = not reported; EO essential oils; TC total cholesterol; HDL-c high-density lipoprotein cholesterol; LDL-c low-density lipoprotein cholesterol

Table 3. *Main bioactive in EOs used in broiler chicken production*

Essential oil	Common name	Bioactive compounds
Allium sativum	Garlic	Allicin, diallyl sulfite
Zingiber officinale	Ginger	Camphene, zingiberene, zingerone, Bisabolene
Thymus vulgaris	Thyme	Thymol, carvacrol, δ-terpinene, ρ-cymene
Origanum vulgare	Oregano	Carvacrol, thymol, γ-terpinene
Rosmarinus officinalis	Rosemary	1,8-Cineole, Camphor, α-Pinene, bornyl acetate
Cinnamomum verum	Cinnamomum	Cinnamaldehyde, linalool and β-Caryophyllene
Mentha piperita	Peppermint	menthol and menthone
Citrus limon	Lemon	Limonene, β-pinene and γ-terpinene

Adapted from Caroprese et al. (2023) and Benchaar et al. (2008)

Antimicrobial effect of EO

Research indicates that birds are often exposed to pathogens, such as *Escherichia coli* and *Salmonella spp*., which may cause digestive tract disorders, resulting in poor nutrient digestion and uptake (Islam et al., 2024). The balance of intestinal microbiota composition (i.e., harmful and beneficial microbes) is vital to maintaining a healthy gut (Elnesr et al., 2020). The antimicrobial properties of EO against a wide range of microbes, including Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria have been

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demonstrated, with some EO demonstrating stronger antimicrobial effect than others (Scicutella et al., 2021; Puvaca et al., 2022). Yang et al. (2019) showed that adding cinnamon EO to chicken diets at 50-800 mg/kg reduced cecal *E. coli* count by 3-72% and increased cecal Lactobacillus by 14 – 70% and Bifidobacterium count by 42-92% when compared to the control (without cinnamon EO) group, suggesting that EO can serve as an alternative to antibiotic growth promoters in chickens. In a similar study, Mehdipour and Afsharmanesh (2018) found that EO supplementation at 200 ppm/kg in chicken feed reduced the ileal coliform count by 16% and increased the ileal Lactobacillus count by 11% when compared with the groups fed antibiotics (virginiamycin). The antibacterial effects of EO on broiler chicken performance may be due to additive antagonistic and synergistic effects of their bioactive compounds (Benchaar et al., 2008; Pirgozliev et al., 2019). Evidence exists that a mixture of thymol and carvacrol presented higher antibacterial activity than either compound alone (Lambert et al., 2001). The antibacterial effect of oregano EO has also been attributed to the additive antibacterial effect of thymol and carvacrol. Elgayyar et al. (2001) reported that *E. coli* O157:H7 was inhibited by oregano oil and its two major bioactive compounds, carvacrol and thymol. It is likely that the bioactive compounds in oregano oil penetrated the *E. coli*'s cell membranes and mitochondria of *E. coli* and altered the membrane-bound electron flow and energy metabolism. This eventually results in the failure of the proton pump and the depletion of the adenosine triphosphate (ATP) pool. Studies have also shown that high levels of EO lead to the breakdown of cell membranes and denaturation of cytoplasmic proteins of bacteria (Nazzaro et al., 2013; Gopi et al., 2014). The use of dietary EO to prevent the growth of yeast, fungi, and molds in the chicken gut and contribute to better gut health has been reported by Esper et al. (2014). Similarly, studies have shown that EO extracted from oregano, mugwort, or fennel can be used to prevent coccidiosis in chickens (Dragan et al., 2014; Murakami et al., 2014).

Antioxidant activity of EOs

Essential oils have been extensively studied for their antioxidant properties in animal nutrition (Puvaca et al., 2022). Antioxidants are compounds that prevent oxidation, a chemical reaction that produces free radicals that can harm cells when their concentrations in the body exceed a certain threshold. Essential oils are rich in antioxidant compounds that, when added to chicken feed, reduce lipid peroxidation in thigh muscles. According to Yang et al. (2019), thigh muscle is susceptible to lipid peroxidation owing to its high content of polyunsaturated fatty acids (PUFAs). Oxidation of PUFAs produces malondialdehyde (MDA), which can be used as a biomarker of lipid peroxidation and oxidation stress (Tongnuanchan and Benjakul, 2014). A study by Keshvari et al. (2013) showed that EO decreased MDA formation and can be blended with other EOs to prevent lipid peroxidation and the deleterious effect of free radicals in the body. Furthermore, Symeon et al. (2014) reported that the addition of 0.5 or 1.0 mL/kg cinnamon EO to chicken diets tended to decrease muscle MDA levels in broiler chickens. The reduction in muscle MDA might be due to the action of cinnamon EO in improving hepatic antioxidant enzyme activities, such as catalase, γ-glutamyl transferase, glutathione peroxidase, or superoxide dismutase (Ciftci et al., 2010). In a similar experiment, Adaszynska-Skwirzynska and Szczerbínska (2019) found that EOs are effective in stopping lipid oxidation, like vitamin E (α-

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tocopherol) or a blend of synthetic compounds [butylated hydroxyanisole BHA) and butylated hydroxytoluene (BHT)].

The enhanced ability of EO from thyme (88.0%), sage (73.9%), and rosemary (78.8%) to prevent oxidation of fatty emulsions has been reported by Adaszynska-Skwirzynska and Szczerbínska (2019). This implies that EO is rich in antioxidative compounds that can be added to animal nutrition to reduce oxidative rancidity in meat (Hashemipour et al., 2013). The use of synthetic antioxidants (i.e., vitamin E and BHT) to reduce lipid peroxidation in chicken meat during cold storage has also been demonstrated (Tongnuanchan and Benjakul, 2014). On the other hand, the oxidative status of broiler meat can be improved using natural vitamin E, thymol, turmeric, cinnamon, oregano, carvacrol, thymol, or rosemary oil (Hashemipour et al., 2013; Adaszynska-Skwirzynska and Szczerbínska, 2019).

Changes in the levels of antioxidant enzymes (e.g., glutathione peroxidase) aid in determining the impact of stress factors, like transportation and high temperature, on the oxidative balance in the animal body (Vosmerova et al., 2010). Evaluation of such indices is now used in testing for new natural antioxidants in chicken feed (Akbarian et al. 2014). In broiler chicken, Akbarian et al. (2014) found that dietary turmeric oil supplementation at 400 mg/kg feed increased the concentrations of antioxidant enzymes under heat stress conditions. This implies that turmeric EO supplementation at 400 mg/kg feed alleviates the stress emanating from an elevated temperature in broiler chickens. Beneficial effects of EOs from oranges, thyme, and others in broiler chickens exposed to heat stress have also been reported by others (Parvar et al., 2013; Gopi et al., 2014; Rimini et al., 2014).

Immunomodulatory effect of essential oils

Dietary EO supplementation may boost the chicken immune system by increasing immunoglobulin (Ig) production, lymphocytic activity, and interferon-γ release (Gopi et al., 2014; Krishan and Narang, 2014). Supplementation of thyme oil at 0.5 g/kg of feed increased the level of IgA in broiler chicken intestine (Puvaca et al., 2022). It has also been reported that the addition of eucalyptus and peppermint EO to water at 0.25 ml/L improved immune responses in broiler chickens (Adaszynska-Skwirzynska and Szczerbínska, 2019). Similarly, Saleh et al. (2014) found that supplementation of thyme and ginger EO at 100 and 200 mg/kg feed increased antibody production. Likewise, Gopi et al. (2014) revealed that EO supplementation relieved the stress caused by vaccination in chickens. According to Yang et al. (2019), incorporating 200 mg/kg cinnamon EO into poultry feed increased serum IgM level on day 42 of age, indicating that cinnamon EO may benefit broiler chicken immune function. In contrast, Huang et al. (2024) found that inclusion of high doses (i.e., 600–800 mg/kg) of microencapsulated EO in broiler chicken feed decreased immune responses. This reduction in immune responses might be related to the ability of EO to inhibit the growth of both beneficial and harmful bacteria in the gut at a higher dose (Abdelli et al., 2021; Greene et al., 2022). The varied responses among animals fed EO products reflect differences in diet composition, quantity of EO included in the diet, type of EO, and chemical structure.

Mechanisms of action of essential oils

Several theories have been proposed to explain how EO exerts its growth enhancement effect in animals. This mechanisms includes: (1) Maintaining healthy and

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balance gut microbiota composition (Yang et al., 2019; Iqbal et al., 2021; Ma et al., 2022) by inhibiting phosphorylation, protein translocation, electron transportation gradients, and other enzyme-dependent reactions in bacterial cell membrane (Benchaar et al., 2008); (2) Changes in gut metabolic processes in favor of the release of gastric juices and digestive enzymes (Xu et al., 2018; Yarmohammadi et al., 2020; Alagawany et al., 2021; Elbaz et al., 2022; Huang et al., 2024); (3) Improvement in feed intake, efficiency of digestion, and nutrient uptake in the gastrointestinal tract (Saleh et al., 2014; Zhai et al., 2018; Elbaz et al., 2022; Puvaca et al., 2022); (4) activation or stimulation of gut defense system through upregulation of immune-related genes and antimicrobial peptides messenger ribonucleic acids (Adaszynska-Skwirzynska and Szczerbińska, 2017; Yang et al., 2019; Huang et al., 2024); (5) improving intestinal permeability and integrity and resistance to pathogen invasion (Fusco et al., 2021; Huang et al., 2024); and increase production of antioxidant enzymes and reduction in cellular damage from free radical attack (Vosmerova et al., 2010; Huang et al., 2024).

Responses of broiler chickens to dietary EO supplementation

Growth performance

The well-reported antibacterial property of EO has led several investigators to ascertain their ability to modulate gut microbiota composition as a way of enhancing growth performance in broiler chickens (Zhai et al., 2018; Yarmohammadi et al., 2020; Puvaca et al., 2022; Huang et al., 2024). Available data indicate that apart from acting as antimicrobial agents, the use of EO in chicken nutrition may be connected to their role in lipid metabolism, release of digestive enzymes, and improvement in gut functions, which all lead to improved BWG (Puvaca et al., 2022). There is growing evidence of the positive effects of EO on the production characteristics of broiler chickens (Bozkurt et al., 2009; Yarmohammadi et al., 2020; Puvaca et al., 2022). Most recently, Huang et al. (2024) showed that inclusion of microencapsulated EO (200–400 mg/kg) in maize-soybean meal-based diets increased BWG in Arbor Acres broiler chickens. In a similar study, Elbaz et al. (2022) found that supplementation of cinnamon EO at the levels of 50, 100, and 200 mg/kg enhanced growth performance. These findings are in harmony with Herve et al. (2019) who found improved growth performance in birds fed ginger EO-supplemented diets at 50, 100, and 150 μl/kg. This improved performance can be attributed to increased intestinal barrier functions after the ingestion of EO. Another possible explanation is the role of EO in encouraging the growth of desirable microbes in the digestive tract (Scicutella et al., 2021; Puvaca et al., 2022). By increasing the proliferation of beneficial microbes in the digestive tract, broiler chickens are less exposed to toxins and undesirable products of microbiological activity, such as ammonia and biogenic amines (Islam et al., 2024). The potential of EO to stimulate the release of digestive enzymes and increase nutrient digestion and absorption may also explain the beneficial impact of EO on BWG in broiler chickens (Yarmohammadi et al., 2020; Puvaca et al., 2022; Huang et al., 2024).

Studies on the effects of EO on FI and FCR were inconsistent, with several authors reporting the positive impacts of EO on ADFI and FCR in broiler chickens (Saleh et al., 2014; Zhai et al., 2018; Puvaca et al., 2022) as displayed in *Table 1*. Mehdipour and Afsharmanesh (2018) revealed that supplementation of EO extracted from cinnamon (*Cinnamomum zeylanicum*) at 200 mg/kg enhanced FCR; however, ADFI was not significantly affected. This is similar to the report of Pathak et al. (2016) in feeding a

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mixture of calcium formate and cinnamaldehyde (500 mg/kg diet) to diseased-challenged Cobb 400 broiler chickens. The improvement in FCR could be due to better efficiency of digestion and nutrient utilization, leading to an increase in BWG of broiler chickens fed EO-supplemented diets. In converse, Yang et al. (2019) found no difference in ADFI and FCR of broiler chickens between the groups offered cinnamon EO at 50-80 mg/kg and the control group. This finding is in harmony with the result of Symeon et al. (2014), who demonstrated that cinnamon EO supplementation at 0.5 and 1.0 ml/kg did not affect the ADFI or FCR of Cobb 700 broiler chickens. This agrees with Huang et al. (2024), who found that EO supplementation in broiler chicken rations at 600 to 800 mg/kg reduced ADFI in Arbor Acres broilers. This could be related to bioactive compounds in the test diets, which may have exceeded a threshold level beyond the tolerance level of experimental broiler chickens. The varied response might be attributed to differences in quantity and type of EO used, diet composition, feeding duration, and health status, all of which affect the effectiveness of EO as growth promoters in broiler chickens.

Blood characteristics

Blood and its metabolites are used as indicators of the internal and external environment of animals (Chowdhury et al., 2018). Blood parameters that are widely used in nutritional assessment are hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), differential WBC, serum protein, glucose, urea, creatinine, lipids [cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), very-low-density lipoprotein cholesterol (VLDL-c), and triglycerides], enzymes (alanine transferase (ALT), aspartate transferase (AST), and alkaline phosphatase (ALP)), minerals, and bilirubin (Ogbuewu and Mbajiorgu, 2024). A recent study by Islam et al. (2024) showed that EO supplementation at 150 mg/kg did not affect PCV, Hb, WBC, and RBC in broiler chickens, which is similar to the results of Toghyani et al. (2010) in broilers fed thyme and ginger oil at 100, 200, and 300 mg/kg feed.

Dietary supplementation of commercially available EO named Activo™ (EW Nutrition Germany) at 0.1 g/kg elevated serum glucose level and antibody titer in Ross 308 broiler chickens at day 35 of age. This contrasted with Islam et al. (2024), who found that EO supplementation (150 mg/kg) had no significant effect on serum proteins but raised ALT and reduced serum ALP content in the broiler chicken sera. In a recent study, Abd EL-Dayem et al. (2024) noticed reduced serum total protein, albumin, and globulin content in diseased-challenged broiler chickens fed thymol or carvacrol at 0.5 ml/L or their blend. In the same study, the authors found that supplementation of thymol or carvacrol at 0.5 ml/L or their blend did not affect serum urea, ALT, or AST in disease-challenged broiler chickens.

Most of the published studies on blood components of broiler chickens fed EO-supplemented diets focus on lipid profiles. The reduction effects of EO on serum cholesterol content in broiler chickens have been demonstrated (Ciftci et al., 2010; Iqbal et al., 2021; Puvaca et al., 2022; *Table 2*). An earlier study by Aghazadeh et al. (2011) revealed that supplementation of broiler chicken diet with thyme EO reduced the levels of triglycerides, cholesterol, HDL-c, LDL-c, and VLDL-c in the serum. In a similar study, Ghazalah and Ali (2008) found incorporation of rosemary into the broiler chickens diet at 0.5 to 2.0% had a significant reduction effect on serum cholesterol content, which is similar to the recent findings of Zhai et al. (2018) and Ciftci et al.

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(2020). The reduction in blood cholesterol levels in broiler chickens may be due to the ability of bioactive substances in EO to inhibit the activities of HMG-CoA reductase and cholesterol-7-hydroxylase fatty acid synthase in the liver (Jazi et al., 2018; Schoeler and Caesar, 2019). This can also be explained by the action of saponins, one of the predominant phytochemicals in EO to form an insoluble complex with cholesterol in the gut, thereby reducing the absorption of cholesterol (Abdulkarimi et al., 2011). The ability of some of the bioactive substances in EO to reduce cholesterol content by upregulating the expression of the 3-hydroxy-3-methyl glutaryl-CoA reductase protein has also been documented (Puvaca et al., 2022).

However, other authors reported that dietary EO supplementation did not reduce blood cholesterol and triglyceride content in broiler chickens (Bolukbasi et al., 2006; Toghyani et al., 2010). This was in harmony with the findings of Saleh et al. (2019) and Iqbal et al. (2021), who stated that EO did not affect serum cholesterol and HDL-c in broiler chickens. Lee et al. (2003) reached the same conclusion after the addition of cinnamaldehyde to the poultry diet, although a hypocholesterolemic effect of EO was discovered by Pathak et al. (2016). Inconsistent findings in serum lipid profiles in broiler chickens fed EO-supplemented diets might be attributed to EO composition, dose rates, diet composition, age or health status of birds, diet composition, hygienic conditions of the rearing environment, and among others (Zeng et al., 2020).

Intestinal histomorphology

Islam et al. (2024) found A positive correlation between enhanced production parameters and intestinal histomorphology development, digestive enzyme activity, and nutrient digestibility in chickens. Intestinal histomorphological parameters [i.e., villus height (VH) and crypt depth (CD)] are essential indicators for assessing the absorptive capacity of the intestine (Iqbal et al., 2021; Ogbuewu and Mbajiorgu, 2024). Lower VH and CD in chickens indicate poor nutrient uptake due to the reduced surface area for absorption. Thus, poor nutrient absorption leads to poor growth performance in chickens (Ogbuewu and Mbajiorgu, 2024). Increased VH in broiler chickens reared under non-challenged or challenged conditions due to dietary EO supplementation has been demonstrated (Yang et al., 2019; Abd El-Hack et al., 2020; Pham et al., 2022; Pham et al., 2023). Broiler chicken fed cinnamon EO (300 mg/kg) had better VH than those offered bacitracin methylene disalicylate (Chowdhury et al., 2018). Iqbal et al. (2021) found improved VH in Ross 308 broiler chickens. Previous trials have shown that EO had a positive effect on duodenal morphology (Zeng et al., 2015; Yang et al., 2019), leading to improved performance and nutrient digestibility (Iqbal et al., 2021). Free radicals released into the gastrointestinal tract during digestion attack the superficial mucous of the intestine and reduce its absorptive capacity (Abd El-Hack et al., 2020). Although the mechanism by which EO increased nutrient utilization in the small intestine is not clear, however, it could be related to the potential of bioactive compounds in EOs to protect the villi from oxidative damage by stimulating the activities of antioxidant enzymes (superoxide dismutase and catalase) and the phenol group (El-Baroty et al., 2010). On the other hand, the effects of EOs on intestinal morphological structures are mediated by their ability to close the tight junctions (Huang et al., 2024), reduce mucosal damage, and increase mucus formation (Pathak et al., 2016), resulting in improved nutrient utilization in the gut and BWG.

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Meat quality and immune organs

The effects of EO supplementation on carcass characteristics (i.e., carcass yield, cut-part weight, and quality) have been investigated, with some reporting positive effect or no effect of EO on carcass parameters (Gomathi et al., 2018; Tekce et al., 2020; Iqbal et al., 2021). Studies have also demonstrated that EO decreases abdominal fat deposition and reduces the content of omega-3 and 6 fatty acids in the liver (Dalkilic et al., 2009), which corroborates Ciftci et al. (2010), who stated that cinnamon EO supplementation in the diet at 500 and 1000 mg/kg feed improves the level of PUFA and omega-6 fatty acids in the blood. However, cinnamon EO supplementation at 500 and 1000 mg/kg feed did not affect the concentrations of serum monounsaturated fatty acids or omega-3 fatty acids but reduced the level of serum saturated fatty acids in chickens. This observation agrees with Puvaca et al. (2022), who reported that EO influenced fat metabolism in poultry by reducing abdominal fat deposition. In converse, Symeon et al. (2014) found that cinnamon EO supplementation at 0.5 or 1.0 mL/kg diet did not affect the carcass traits and the visceral organ weights in broiler chickens, which is similar to the report of Devi et al. (2018), who stated that addition of cinnamon EO to poultry feed at the rates of 3 and 4 g/kg did not affect carcass yield and quality. In another study, Gomathi et al. (2018) and Ghazanfari et al. (2024) discovered that abdominal fat, carcass yield, and aspects of visceral weights were not affected by EO supplementation in broiler chickens. These findings are consistent with those of Symeon et al. (2014), who stated that EO supplementation at 0.5 or 1.0 ml/kg diet had no statistical effect on pH24 (i.e., pH of meat 24 h after slaughter), color parameters, cooking loss, or shear force value of broiler chicken breast muscle. The observed disparity among studies that assessed the impact of EO on carcass traits in broiler chickens could be due to a variety of factors, such as diet composition, chicken age, level and type of EO used, and so on.

Immune organs (Bursa of Fabricius, spleen, and thymus) are important for chickens, and their status is closely connected with immune functions. Yang et al. (2019) stated that the weight of immune organs could be employed to assess the immune status, and elevated weights of immune organs usually connote stronger immune functions to some extent. A study by Ghazanfari et al. (2024) revealed that dietary EO supplementation at 150 mg/kg had no effect on the weight of Bursa of Fabricius, which is in harmony with the findings of Toghyani et al. (2011), who discovered that the diet incorporating cinnamon EO at 2 or 4 g/kg did not affect the weights of Bursa of Fabricius and spleen in broiler chickens.

Constraints to the use of EO in broiler chicken production

Despite EO’s ability to enhance production characteristics and health status of broiler chickens, there are still limitations to its use in animal nutrition. With more than 3000 identified EO, investigating their individual effects on chicken performance, and health is a challenging task. Further to this, numerous studies used blends of EO, making it hard to know which EO elicited the observed response or if it is necessary to feed the oils together to induce the desired response. Since EO is very expensive, it is important to ascertain the dose levels of EO that optimize animal health, performance, and economic gains to avoid wastage. To date, several EOs, such as capsaicin, garlic, cinnamaldehyde, thymol, carvacrol, and eugenol are well documented in broiler chickens, while others are rarely studied. Without a detailed understanding of the impacts of EO on broiler chicken health and performance and the EO’s mechanism of

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action, it is challenging to provide a proper clue for using EO as growth promoters in animals. More research is required to understand the potential of EO to improve chicken productivity and health.

Conclusion

Taking the inconsistent findings on the impact of dietary EO supplementation on broiler chicken performance into consideration, it is concluded that EO can be added to the broiler chicken diet at moderate levels to improve growth and productivity. The potential of EO to increase broiler chicken performance might be related to one or more of the following mechanisms of action: (i) modulating gut microbiota composition in favor of the growth of healthy microbes through bactericidal or bacteriostatic effects; (ii) changes in gut metabolic processes in favor of the release of gastric juices and digestive enzymes; (iii) improving feed intake, digestion, and nutrient uptake in the gastrointestinal tract; (iv) activation or stimulation of gut defense system through upregulation of immune-related genes and antimicrobial peptides messenger ribonucleic acids (mRNA); (v) improving intestinal permeability and integrity and resistance to pathogen invasion, and (vi) increase production of antioxidant enzymes and reduction in cellular damage from free radical attack. However, as with other novel feed additives, the use of EOs in the poultry industry owing to inconsistent results in chickens fed EO-supplemented diets is driven by factors such as part of the plant used, age of the plant, level, and type of EO used, and several others. As a result, further studies are required using meta-analysis and a quadratic optimization model to determine the best supplementation levels of EOs that significantly improve the performance indices of broiler chickens. The use of transcriptomics to understand the effect of EOs on the expression of growth genes in broiler chickens is recommended as such data is scarce in the literature.

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