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REVIEW

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Thymol and carvacrol supplementation in poultry health and performance

Majid Gholami-Ahangaran[1] | **Asiye Ahmadi-Dastgerdi**[2] | **Shahrzad Azizi**[3] | **Asal Basiratpour**[4] | **Maryam Zokaei**[5] | **Masoud Derakhshan**[6]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

[2] Department of Food Science and Technology, Ardestan Branch, Islamic Azad University, Ardestan, Iran

[3] Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

[4] Graduated of Basic Sciences Faculty, Payame Noor University, Isfahan Branch, Isfahan, Iran

[5] Department of Food Science and Technology, Faculty of Nutrition Science, Food Science and Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[6] Department of Veterinary Medicine, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

Correspondence
Majid Gholami-Ahangaran, Department of Clinical Sciences, Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, P.O. Box:166, Shahrekord, Iran.
Email: mgholami6@gmail.com

Abstract

Background: Thymol and carvacrol as natural essential oils and phenol compounds are components derived from some medicinal plants, such as thyme and oregano species.

Objectives: The increasing demands in organic and healthy meat and egg consumption in human society have made it necessary to consider alternative natural compounds for the replacement of chemical compounds in poultry production. The chemical compounds can remain in meat and eggs and cause complications in human health. Therefore, these natural compounds can be fed with a higher safety in poultry production with specific effects. In this regard, the role of thymol and carvacrol as natural compounds in the poultry production has been discussed in the review.

Methods: In this study, by searching for keywords related to thymol and carvacrol in poultry production in Google Scholar database, the articles related to different aspects of the biological effects of these two phytogenes in poultry production were selected and analyzed.

Results: A review of previous studies has shown that thymol and carvacrol possess a wide range of biological activities, including antibacterial, antiviral, antioxidant, anti-inflammatory, modulating of immunity response and regulating of the gut microbial population. Also, in meat type chickens can promote growth and influence feed utilization. The beneficial effect of this compound was evaluated in hepatic toxicity and demonstrated as a hepatoprotective compound in chickens. Furthermore, these compounds can affect the behavior of layers and influence egg composition, eggshell thickness, and the sensory quality of eggs.

Conclusion: It seems that with the increasing demand for healthy protein products, these compounds can be used to improve performance as a substitute alternative for chemical compounds in healthy poultry farms.

KEYWORDS
carvacrol, poultry, production, thymol

1 | INTRODUCTION

In recent years, following the ban on the use of antibiotics as growth promotors in most countries, non-antibiotic substances with this potential have been considered as effective alternatives comprising probiotics, organic substances, enzymes and phytogenic compounds (da Silveira Deminicis et al., 2021; Ghasemian et al., 2021). The reason for this prohibition was the widespread development of resistant bacterial strains to evolve over a short period and transfer resistance to other strains of bacteria populations (Gholami-Ahangaran, Moravvej et al., 2021; Rahimi et al., 2012).

Phytobiotics, also referred to as phytochemicals or phytogenics, are a broad subset of plant-derived bioactive compounds. Thus far, more than 8000 individual dietary phytobiotics have been identified in fruits, vegetables, whole grains, legumes, nuts and herbs so far like phenols, flavonoids, tannins, saponins, essential oils and so forth (Yadav et al., 2016). They can be added to the diet of commercial animals to improve their productivity through enhancing feed properties, promoting animals' production performance and improving the quality of products derived from these animals (Gholami-Ahangaran, Karimi-Dehkordi, et al., 2021).

The shift towards herbal medicine in recent years is more due to the advantages of these over chemical drugs that include reduced or zero toxicity and are available naturally and possess ideal qualities as feed additive (Gholami-Ahangaran, Haj-Saleh, et al., 2021). The products derived from plant parts, specifically essential oils, are known to possess active ingredients that exhibit antimicrobial activity against bacteria, yeast and molds (Bahmani et al., 2014). Among the major groups of principle ingredients that impart antimicrobials property in their essential oils include thymol, eugenol, saponins, flavonoids, carvacrol, terpenes and their precursors (Gholami-Ahangaran et al., 2020). Phytobiotics can be divided into the following six categories: phenolic compounds, alkaloids, nitrogen-containing compounds, organosulfur compounds, phytosterols and carotenoids, and they are further divided into several subcategories. There have been several investigations on phenolic compounds and carotenoids to determine their biological effects and characteristics (Kikusato, 2021).

Natural plant products may have a variable composition of individual compounds. For example, the percentage of the two main constituents of thyme essential oils, that is, thymol and carvacrol, can vary from as low as 3% to as high as 60% (Gholami-Ahangaran et al., 2019). Similar variation can be found also in the essential oil of oregano (*Origanum vulgare* ssp. *hirtum*), which is obtained by steam-distillation of the plant that contains more than 30 bioactive components, most of which are phenolic substances with varying activities. Major components are carvacrol and thymol, which represent over 80% of the total oil, with carvacrol being the main component in most cases, although sometimes it can be found only in traces (Skoufos et al., 2020). The content of active substances and the chemical composition of phytobiotics in the final products may vary widely depending on the plant parts used (seeds, leaves, etc.), geographical origins and harvesting season. It has also been suggested that the benefits of the use of essential oils of oregano are often also variable because it depends on all the constituents working together (Ghasemian et al., 2021).

In the last two decades, phytobiotics have been shown to exert multiple effects, including anti-inflammatory, antimicrobial, anti-oxidative and metabolic-modulating effects. Phytobiotics are used for promoting growth and improving meat and egg quality in poultry production (Kikusato, 2021). Although, the exact mechanism of action is not yet known, they have been found to favourably alter the gut microflora by reducing the number of pathogenic organisms. The probable mechanism of action is through the alteration in membrane permeability to hydrogen ions (Gholami-Ahangaran et al., 2020).

Phytochemicals possess antioxidants (both hydrophilic and lipophilic activity). Due to their antioxidant activity, these compounds are being used during stress periods including heat stress conditions. Their antioxidant property could be helpful in improving the keeping quality of processed meats and also reduces the muscle drip loss during thawing of cold-stored products (Yadav et al., 2016). Furthermore, some studies have shown that phytobiotics can enhance the digestive enzyme activity and absorption capacity. In addition, the results of some studies have demonstrated that phytogenic products may be able to stimulate intestinal mucus production that may further contribute to the relief from pathogen pressure through inhibiting adherence to the mucosa (Mohammadi Gheisar & Kim, 2018).

In this review, I focused on the effects of thymol and carvacrol and the possible mechanisms underlying their overall effects on gastrointestinal function, immunity response, inflammatory status, production and metabolism rather than the specific effects of each compound. We also discuss the possible mechanisms by which thymol and carvacrol contribute to growth promotion in poultry. In the current review, we try to discuss the earlier documents on the effectiveness of thymol and carvacrol in healthy poultry performance.

1.1 | Thymol

Thymol ($C10H14O$) is a monoterpene that is found in many plant oils. Thymol is a natural phenol compound that is known as 2-isopropyl-5-methyl phenol and is a colourless crystalline monoterpene phenol (Table 1). It is one of the most important dietary constituents in thyme species (Attia et al., 2017; Bahmani et al., 2014). For centuries, it has been used in traditional medicine and has been shown to possess various pharmacological properties including antioxidant, anti-inflammatory, antibacterial, analgesic, antispasmodic, antifungal, antiseptic and antitumor activities. Also, thymol possesses multiple therapeutic effects against various cardiovascular, gastrointestinal, neurological, rheumatological, metabolic and malignant diseases (Attia et al., 2018; Gholami-Ahangaran et al., 2020). The effects of thymol are largely attributed to its anti-inflammatory (via inhibiting recruitment of pre-inflammatory cytokines and chemokines), antioxidant (via scavenging of free radicals, enhancing the endogenous enzymatic and non-enzymatic antioxidants and chelation of metal ions), anti-hyperlipidemic (via increasing the levels of high density lipoprotein

TABLE 1 Chemical properties of the thymol and carvacrol (Lee, 2002)

Chemical property	Thymol	Carvacrol
Molecular weight	150 $C_{10}H_{14}O$	150 $C_{10}H_{14}O$
Synonym	5-methyl-2-(1- methylethyl)phenol	2-methyl-5-(1- methylethyl)phenol
FEMA-GRAS (Flavor and Extract Manufacturers Association-Generally Recognized As Safe)	3066	2245
FDA (Food and Drug Administration)	21CFR 172.515	21CFR 172.515
Appearance	White crystals	Colourless to pale yellow liquid
Odour	Pungent, caustic taste	Thymol odour-like
Boiling point	233	237
Density (g/Ml)	0.969	0.976
LD_{50}	980 mg/kg, orally rat	810 mg/kg, orally rat
Stability	Good	Good

TABLE 2 The main effect and mechanism of action of thymol

Effects	Mechanism of action	References
Anti-inflammatory	Inhibiting recruitment of pre-inflammatory cytokines and chemokines	Wechsler et al. (2014)
Antioxidant	Scavenging of free radicals, enhancing the endogenous enzymatic and non-enzymatic antioxidants and chelation of metal ions	Nagoor Meeran et al. (2017)
Anti-hyperlipidemic	Increasing the levels of HDL cholesterol and decreasing the levels of LDL in the circulation and membrane stabilisation	Luna et al. (2018)

(HDL) cholesterol and decreasing the levels of low density lipoprotein (LDL) in the circulation and membrane stabilisation) effects. It is one of the potential candidates of natural origin that has shown promising therapeutic potential (Gholami-Ahangaran et al., 2015; Table 2).

Thymol is abundantly found in certain plants such as *Thymus vulgaris*, *Ocimum gratissimum*, *Thymus ciliates*, *Satureja thymbra*, *Thymus zygis*, *Trachyspermum ammi*, *Carum copticum*, *Satureja intermedia*, *Thymbra capitata*, *Lippia multiflora*, *Thymus pectinatus*, *Satureja hortensis*, *Centipeda minima* and *Nigella sativa* seeds (Nagoor Meeran et al., 2017). Also, thymol is a component derived from some medicinal plants, such as *Monarda fistulosa*, *Origanum compactum*, *O. dictamnus*, *O. onites*, *O. vulgare*, *T. glandulosus* and *T. hyemalis* (Abd El-Hack & Alagawany, 2015). Also, the bee balms *Monarda didyma* and North American wild flowers are natural sources of thymol molecule (Tilfort, 1997). Furthermore, *Zataria multiflora* is a medicinal plant belonging to the Lamiaceae family that geographically grows only in Iran, India, Pakistan and Afghanistan. The main constituents of the essential oil of this plant are phenolic compounds such as carvacrol (59%) and thymol (39%). The other source of thymol is presented in Table 3. Thymol molecule is a natural phenol as monoterpene derivative of cymene and an isomer of carvacrol component (Gholami-Ahangaran et al., 2020; Figure 1).

1.2 | Carvacrol

Carvacrol (C10H14O) is a liquid phenolic that is known as 2-methyl-5-(1-methyl ethyl) phenol (Table 1). It is mostly present in oil of oregano (*O. vulgare*), thyme (*T. vulgris*), pepperwort (*Lepidium flavum*), wild bergamot (*Citrus aurantium* var. bergamia Loisel) and other plants. Commercial carvacrol is synthesised by chemical methods (Yadav & Kamble, 2009). It is also named 5-isopropyl-2-methylphenol by the International Union of Pure and Applied Chemistry. This compound possesses a wide range of biological activities, including antibacterial and antifungal (Chavan & Tupe, 2014), antiviral (Sánchez et al., 2015), antioxidant, modulating of immunity response (Khazdair et al., 2018), anti-inflammatory (Fitsiou et al., 2016) and anticarcinogenic properties (Özkan & Erdoğan, 2011). The biological activity and mode of action of carvacrol are presented in Table 4. Due to the flavouring and antimicrobial activities, it is mostly used in the food industry as a natural food preservative (Salehi et al., 2018).

Carvacrol is a component of some medicinal plants, such as black cumin (*N. sativa*), oregano (*O. compactum*), *M. didyma*, *O. dictamnus*, *O. microphyllum*, *O. onites*, *O. scabrum*, *O. vulgare*, thyme (*T. glandulosus*), savoury (*S. hortensis;* Attia & Al-Harthi, 2015; Figiel, 2010). Also,

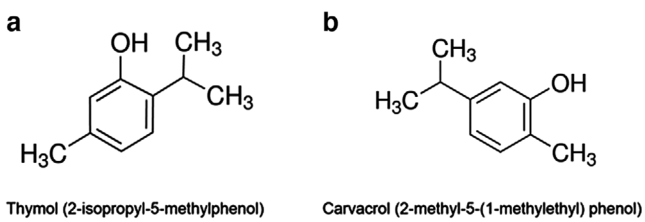

FIGURE 1 Chemical structures of (a) thymol and (b) carvacrol

TABLE 3 Examples of other plant sources of thymol (Salehi et al., 2018)

Family of plants	Plant	Part	Thymol in essential oils (%)	Origin
	Thymus zygis	Aerial parts	15.5–21.0	Portugal
	T. glandulosus	Whole plant	43.2	Morocco
	T. hyemalis	Aerial parts	16.09–29.27	Spain
	T. broussonetii	Whole plant	36.7	Morocco
	Monarda fistulosa	Aerial parts	0.2–12.6	North America/Russia
	M. punctata	Flowers	75.2	China
	M. bradburiana	Aerial parts	57.7	North America
	Origanum dictamnus	Aerial parts	0.13–0.61	Greece
	O. onites	Aerial parts	0.7	Greece
	O. vulgare	Aerial parts	13.7	Greece
	Satureja spicigera	Aerial parts	35.1	Iran
	S. intermedia	Aerial parts	32.3	Iran
	S. mutica	Aerial parts	26.5	Iran
	S. sahendica	Aerial parts	19.6–41.7	Iran
	Zataria multiflora	Aerial parts	39	India, Pakistan
Apiaceae	*Trachyspermum copticum*	Fruits/seeds	72.3/37.2	Iran
	Lagoecia cuminoides	Aerial parts	72.8–94.8	Turkey
Verbenaceae	*Lippia multiflora*	Leaves	14	Africa
	L. gracilis	Leaves	3.83–55.50	Brazil

TABLE 4 The effect and mode of action of carvacrol

Effects	Mode of action	References
Antimicrobial	Interact with the cell membrane by hydrogen bonding, rendering the membranes and mitochondria more permeable and disintegrating the outer cell membrane	Di Pasqua et al. (2010)
Antioxidant	Scavenging of free radicals, inhibit prostaglandin synthesis and induct drug-metabolising enzymes. The reaction of carvacrol with a free radical is facilitated due to its weak acid character, so donating hydrogen atoms to an unpaired electron, producing another radical that is stabilised by electron scattering generated at a molecule resonance structure	Alagawany et al. (2015)
Immunomodulatory	Acting as antioxidants and extending the activity of vitamin C. Improvement in the immune responses of chicks because of the assured antioxidant, antibacterial and antiviral activities of carvacrol.	Alagawany et al. (2015)
Anti-carcinogenic	Genomic DNA fragmentations and caspase-3, -6 or -9 enzymes gene expression were induced by carvacrol; also carvacrol induces apoptosis regulatory genes in human cancer and retarded growth	Zeytun and Ozkorkmaz (2021)
Anti-inflammatory and anti-hypernociceptive	Inhibit the migration of mononuclear cells and neutrophils concluded in the production of pro-inflammatory cytokines such as nitric oxide and consequently a decrease in prostaglandins. Carvacrol decreases the levels of enzyme responsible for inducing nitric oxide synthase and in turns the macrophages content of nitric oxide.	Guimarães et al. (2010)
Anti-obesity	Carvacrol decreased the expression of adipogenesis related genes fibroblast growth factor receptor in visceral adipose tissues. Also, carvacrol decreased the expression of receptors which stimulates the intake of fat-rich diet such as galanin receptors 1 and 2.	Alagawany et al. (2015)

carvacrol has been produced by chemical and biotechnological synthesis via metabolic-engineered microorganisms (More et al., 2007).

1.3 | Biological activity and mode of action

Many biological activities and mechanisms of action of herbal plants like thymol and their products comprise producing desired effects on feed utilisation, nutrient bioavailability, improving digestive enzymes secretion and increasing the motility of the digestive tract, also possessing anti-inflammatory, antispasmodic and antioxidant activities (Alavinezhad & Boskabady, 2014), besides endocrine and immune stimulation, as well as a modifier of gut microbial fermentation and reduction of odour, urea and methane emission (Akyurek & Yel, 2011; Varel & Miller, 2001). Potent antimicrobial action has been documented in vitro and/or in vivo against several pathogens (bacterial, viral, fungal and parasitic), including drug-resistant bacterial strains like methicillin-resistant *Staphylococcus aureus*. These have been proven to be useful antileishmanial, anthelminthic and coccidiostat agents (Gholijani et al., 2015; Gopi, 2014). In order to suppress the inflammation, thymol inhibits the release of neutrophil elastase enzyme in humans (Braga et al., 2006). Thymol is capable of reducing the secretion of enterotoxins A, B and α-haemolysin produced by *S. aureus* isolates (Qiu et al., 2010). Due to its promising potential, thymol can be explored as a new and novel antileishmanial medicinal molecule (Robledo et al., 2005).

Biocide is a concept meaning naturally occurring antimicrobial class of compounds. Thymol is a part of biocides having a strong antimicrobial activity if used alone or mixed with other biocides. Bacterial resistance to common drugs such as penicillin and other antibiotics could be depressed by thymol through the synergistic effect (Nostro et al., 2004). Thymol has been shown to possess antimicrobial effects and powerful antioxidant properties (Zarrini et al., 2010) and could induce antibiotic susceptibility in drug-resistant pathogens. Aijaz et al. (2010) reported a strong antifungal activity of thymol, especially against fluconazole-resistant *Candida albicans* strains (Guo et al., 2009). Thymol as essential oil plays an effective role as an antimutagenic agent (Mezzoug et al., 2007). Furthermore, Trombetta et al. (2005) and Andersen (2006) documented the antitumour activity of thymol. Thymol could act as a positive allosteric modulator of GABAA in vitro (García et al., 2005). Alavinezhad & Boskabady (2014) reported that *C. copticum* L., the source of thymol, has various therapeutic effects against fatigue, bloating, abdominal tumours, diarrhoea, abdominal pain, loss of appetite and respiratory distress. It has additional activities as antioxidant, antiparasitic, hypolipidemic and antibacterial. The nephroprotective effect of thymol may be due to its anti-inflammatory, antioxidant and antiapoptotic properties (Alavinezhad & Boskabady, 2014).

Good knowledge of the carvacrol mode of action is very required regarding application in nutritional systems. Formerly, Ultee et al. (1999) reported the antimicrobial activity of carvacrol on the pathogen *Bacillus cereus*. Carvacrol is a hydrophobic compound and has an effective impact on biological membranes. The modes of action and beneficial aspects of carvacrol are shown in Table 2. Most applications of thymol and carvacrol with major physiological responses in poultry were presented in Table 5.

1.4 | Antibacterial activity

The antimicrobial activity of essential oils has been extensively investigated in several in vitro studies that showed that carvacrol and thymol possess strong antimicrobial effects against pathogenic bacteria such as *Escherichia coli* and *Salmonella typhimurium*, both of which are potential risk factors of enteric infections (Gholami-Ahangaran et al., 2020). The minimum inhibitory concentration (ppm) of thymol and carvacrol is presented in Table 6. Carvacrol, thymol and eugenol-exerted potent antimicrobial activity have similar structures, synergistically bacteriostatic and bactericidal activity, even in lower concentrations ((Bassolé & Juliani, 2012). Therefore, it is necessary to optimise their formulation.

Both carvacrol and eugenol showed potent antimicrobial activity against several microbes such as *S. aureus*, *Clostridia* spp., *E. coli* and *S. pullorum* (Dahiya et al., 2006). Carvacrol and thymol-rich essential oil showed higher inhibitory activity, compared to linalool against avian *E. coli* (Peñalver et al., 2005).

Thymol, carvacrol and eugenol mixtures, astaxanthin isolated from microalgae *Haematococcus pluvaris* and Lupulone were effective in controlling *Clostridium perfringens* colonisation and proliferation in the guts of broilers (Siragusa et al., 2008).

Carvacrol also acts as an antibacterial by preventing the synthesis of flagellin, causing bacteria to be nonmotile (Burt et al., 2007). Antiflagellate activity on *Tetratrichomonas gallinarum* and *Histomonas meleagridis* by essential oils obtained from *Cinnamomum aromaticum* leaves, *Citrus limon* pericarps and *Allium sativum* bulbs were also reported (Burt et al., 2007).

The essential oils used in poultry diets either individually or in combination or solely pure compounds have shown a strong inhibitory effect on *C. perfringens* and *E. coli* in the digestive tract and ameliorated intestinal lesions and weight loss than the challenged control birds (Jerzsele et al., 2012). Combination of oregano essential oil with antibiotics to treat *E. coli* bacteria characterised as extended-spectrum beta-lactamase (ESBL)-producing bacteria. Oregano in combination with antibiotics lowered the effective dose of antibiotics, thus minimising their adverse effects on ESBL-producing *E. coli* (Gholami-Ahangaran et al., 2020).

Carvacrol, cinnamaldehyde, oregano oil and thymol were also found to inhibit *C. perfringens* spore germination and growth in ground turkey during chilling (Juneja & Friedman, 2007). Carvacrol, thymol, trans-Cinnamaldehyde and tetrasodium pyrophosphate on the radio sensitistion of *E. coli* and *S. typhi* in chicken breast significantly reduced the numbers of these pathogens (Lacroix & Chiasson, 2004).

It is well known that the mechanism of antibacterial activity of herbal constituents is linked to their hydrophobicity, which disrupts the permeability of cell membranes and cell homeostasis with the consequence of loss of cellular components, and even cell death

TABLE 5 Application of thymol and carvacrol with major physiological responses in poultry

Compounds	Study design	Main findings	Reference
Essential oil (thymol and cinnamaldehyd)	Type: Ross broilers; dose: 0.1 g/kg (15 g/t thymol and 5 g/t cinnamaldehyde), source: commercial blend oil form: powder duration: 0–42 days	• Higher ADG • Higher number of *Lactobacillus* and *Escherichia coli* in the cecum • Higher proportion of caecal butyrate • Decreased proportion of caecal acetic acid and propionic acid	Xing et al. (2019)
Thymol and carvacrol	Type: layer (Bovans-White), dose: 1000 mg/kg each, source: Thymbra spicata and *Rosemarinus officinalis* extract, form: dry leaf powder, duration: 48–56 weeks	• Lower total cholesterol (TC) and Triglyceride (TG) in serum • Lower egg production and egg weight • No effects on FCR, eggshell thickness, yolk colour and haugh unit	Çimrin (2019)
Thymol	Type: layer (Hi-sex Brown), source: *T. vulgaris* dry herb, form: powder, dose: 3, 6 and 9 g/kg, duration: 36–52 weeks	• No effects on feed intake (FI), ADG and FCR • Higher egg weight • Higher Superoxide dismutases (SOD), Glutathione peroxidase (GSH-PX) and lower MDA in serum • Higher IgG in serum • Lower LDL-C in serum	El-Hack and Alagawany (2015)
Essential oil mixture (carvacrol, thymol, 1:8-cineole, p-cymene and limonene)	Type: ATAK-S laying hens dose: 0, 3 and 6 mg/kg source: commercial form: powder duration: 52–68 weeks	• No effects on FI, egg production, egg weight and FCR • No effects on glucose, TC and TG in serum • No effects on antibody titers against Newcastle disease virus (NDV), infectious bronchitis (IB) a nd infectious bursal disease virus (IBDV)	Özek et al. (2011)
Thymol	Type: quail (female) dose: 2 g/kg (80 mg/bird per day); source: commercial form: powder duration:100–130 days	• No effects on corticosterone concentrations in plasma • Higher albumen, glucose, globulins, TP in plasma • Higher inflammatory responses • Higher heterophil to lymphocyte ratio in blood	Nazar et al. (2019)
Thymol	Type: quail dose: 0, 2, 4 and 6.5 g/kg; source: commercial form: powder duration: 85–128 days	• Lower saturated fatty acids (SFA) in egg yolk • Higher Polyunsaturated fatty acids (PUFA) in egg yolk • No effects on body weight gain, FI, egg production, and egg weight	Fernandez et al. (2019)
Essential oil (oregano oil)	Type: duckling (Cherry valley) dose: 150 and 300 mg/kg; source: commercial-oregano (5% thymol, 65% carvacrol and 30% carrier) form: powder duration:11–42 days	• No effects on final body weight, ADG, FI, and FCR • Lower number of coliform bacterial in the cecum • No effects on TC, TG, aspartate aminotransferase (AST), alanine transaminase (ALT), glucose and total protein (TP) in serum	Abouelezz et al. (2019)
Thymol	Type: turkey poults dose: 30 mg/kg; source: commercial extract form: powder duration:180–236 days	• Improved FCR • No effects on ADG, lactic acid bacteria and coliform count in crop, ileum and cecum • Lower MDA level in liver, thigh and breast muscle • Higher GSH-Px, Glutathione S-transferase (GST) activities in liver, thigh and breast muscle	Giannenas et al. (2014)

TABLE 6 Minimum inhibitory concentration (ppm) of thymol and carvacrol (Lee, 2002)

Microorganisms	Carvacrol	Thymol
E. coli	450	450
E. coli	225	225
Staphylococcus aureus	450	225
Candida albicans	150	150
C. albicans	113	113
C. albicans	200	Not tested
Pseudomonas aeruginosa	500	500
P. aeruginosa	>900	>900
S. typhimurium	150	150
S. typhimurium	225	56
Streptococcus mutans	125	250
S. mitis	125	125
E. coli	450	450
E. coli	225	225

(Solórzano-Santos & Miranda-Novales, 2012). Plant essential oils and their components are mostly hydrophobic. Due to the hydrophobicity of essential oils, they are partitioned between the lipids of the cell membrane and mitochondria rendering them permeable and causing the leakage of cell contents, leading to cell death (Burt, 2004). This property enables the herbal compounds to penetrate the lipid layers in the bacterial cell wall and mitochondria and disturb the cell structure. Cowan (1999) reported that 60% of essential oil derivatives examined were inhibitory to fungi while 30% inhibited the growth of bacteria. Usually, the essential oils contain a high level of phenolic components, including carvacrol, and thymol and eugenol have the strongest antibacterial properties against food-borne pathogens. Both the phenolics carvacrol and thymol are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to Adenosine triphosphate (ATP) and depolarise the cytoplasmic membrane (Xu et al., 2008). Helander et al. (1998) investigated the antimicrobial mechanism of the two isomeric phenols, carvacrol and thymol and one phenylpropanoid, cinnamaldehyde, on *E. coli* and *S. typhimurium*. It was observed that both carvacrol and thymol disintegrated the outer bacterial membranes in a similar manner, thus intracellular material from cells is transferred to the external medium due to membrane disruption. On the other hand, cinnamaldehyde did not affect the membrane but showed potent inhibitory antibacterial activity. It was suggested that the phenylpropanoid cinnamadlehyde penetrates the bacterial membrane and thus can reach and affect the inner part of the cell. In agreement with the previous findings, it has also been hypothesised that whole essential oils have greater antibacterial activity than their major components (e.g., solely carvacrol), and this suggests that the minor components in essential oils are also main to the activity and may have a synergistic effect (Brenes & Roura, 2010). The two major components of oregano essential oil, carvacrol and thymol, were found to give a synergistic effect when tested against *S. aureus* and *P. aeruginosa* (Lambert et al., 2001). The synergic effect between carvacrol and its biological precursor p-cymene has been observed. Although p-cymene is a weak antibacterial substance, it can swell bacterial cell membranes to a larger degree, compared to carvacrol. In this way, p-cymene may enable carvacrol to be more easily transported into the cell so that a synergistic effect is achieved. However, a bacteriostatic or a bactericidal effect of plant extracts, essential oils and herbal compounds is lower than antibiotics (Florou-Paneri et al., 2005). However, the effective levels of those compounds are very higher than the cost-effective levels in animal production. It should not be overlooked that these compounds can be useful in breeding organic and healthy chickens that are not allowed to use antibiotics and chemical compounds.

1.5 | Antiviral activity

Herbal plants and their derivatives or extracts have been evaluated for their possible antiviral prosperities, including the cold-pressed or essential oils of certain commonly used culinary herbs (Jassim & Naji, 2003; Sokovicxét al., 2010). Carvacrol plays a key role as an antiviral component against human rotavirus (RV). In the same context, Mexican oregano (*L. graveolens*) extract and oil as well as carvacrol components are able to reduce/inhibit viral diseases in animals and humans. Specifically, the antiviral activity of oregano and its phenolic components on acyclovir-resistant herpes simplex virus type 1 and human respiratory syncytial virus and carvacrol on RV have been documented (Bernstein, 2009; Pilau et al., 2011). Oregano essential oils including carvacrol compound have been strongly promoted as natural antiviral factors effective against many viral diseases such as the pandemic H1N1 influenza virus (Vimalanathan & Hudson, 2012). On the contrary, Sökmen et al. (2004) noted that anti-influenza virus activity was not affected by the supplementation of oils or extracts derived from oregano. Gilling et al. (2014) reported that carvacrol as a natural compound is very effective in inhibiting human norovirus within 4 h of exposure by acting directly on the viral capsid.

1.6 | Growth performance

Aromatic medicinal plants and their extracts are claimed to be beneficially effective on the flavour and palatability of feed, thus enhancing the growth and performance (Windisch et al., 2008). It has been stated that dietary supplementation with herbal products has growth-promoting effect on broiler chickens (Gholami-Ahangaran et al., 2020). One study stated that carvacrol reduced feed intake (FI), weight gain and feed conversion rate (FCR), and thymol showed no effect when broilers were fed with 200-mg/kg carvacrol and thymol (Lee et al., 2003).

While Yakhkeshi et al. (2011) studied the effects of different natural growth promoters in broiler chickens to compare the results, it was found that feeding the birds with diets containing natural feed additives instead of antibiotics alleviated the negative effects of removing antibiotics from the diet of commercial poultry. Cross et al. (2007) found an increase in body weight gain of broiler chickens diet supplemented with thyme essential oil at a level of 1000 mg/kg in spite of decreased FI by nearly 10%. Hernández et al. (2004) supplemented broiler diets with different additives involved with thymol, sage and rosemary extracts and peppers essential oil and examined the effect of these additives on feed digestibility and reported an improvement in digestibility and final productive performance.

However, it is hard to find the negative effects of plant extracts on poultry performance. It seems, their effectiveness is inconstant due to some factors, such as the type of essential oil, age of birds, the composition of diets and environmental factors (Zhang et al., 2014).

There are many different types of essential oils known worldwide (about 3000), of which about 300 exhibits commercial interest (Brenes & Roura, 2010). Among these essential oils, extracts of oregano, rosemary, thymol, cinnamon and coriander have been tested in poultry (Windisch et al., 2008). These essential oils vary in composition and level of active substances due to many reasons (Brenes & Roura, 2010). As reviewed in the literature, the growth performance in poultry varies with different essential oil sources and dosage levels (Brenes & Roura, 2010; Giannenas et al., 2018; Lee et al., 2004). As a result, sometimes, inconsistent results that were reported by several researchers in vivo could be related to experimental design, formulation of basal diets, and it could also be largely attributed to the dose and duration of the consumption, composition and concentrations of the included active substances. In addition, the concentration of the bioactive compounds related to extraction methods and the origin of the plant materials used, as well as the cultivation parameters. Therefore, essential oils from the same plant species that are used in different trials may differ in composition and activities. For example, the main active substances that have antimicrobial functions in oregano essential oils are carvacrol and thymol, which content may differ as largely as 5% to 85% (Burt, 2004). Moreover, it is known that there are 60 plants with the same name of 'oregano' in Europe (Giannenas et al., 2018; Lawrence& Reynolds, 1984). Among them, Greek oregano (*O. vulgare* L. synonym *O. heracleoticum* ssp. *hirtum*) has the highest content of the antimicrobial active substance carvacrol. The studies of thymol and carvacrol effects on growth performance in poultry are listed in Table 7.

1.7 | Feed utilisation

Essential oils have an important impact on FCR, as they benefit microorganism population stability and augment nutrient absorption. In addition, essential oils can activate enzymes and enhance digestion (Youssef et al., 2021). Lee et al. (2003) found that carvacrol and thymol improved FCR in broilers, with the inactivation of insulin sites in the liver. This led to increased efficiency, manifested as feed utilisation. Furthermore, these oils help protein digestibility by alleviating pepsin and Hydrogen chloride (HCL) secretion (Gopi, 2014; Mathlouthi et al., 2012). Fotea et al. (2010) studied the effect of oregano oil (0.3%, 0.7% and 1%) and showed that 1% levels improved FCR. Roofchaee et al. (2011) found that the addition of 600 mg/kg of oregano oil in diets significantly decreased FI and improved FCR. Esper et al. (2014) examined Gas chromatography–mass spectrometry (GC/MS) analysis for *O. vulgare* components and showed that the essential oil contained 4-terpineol that inhibited aflatoxins. These results indicated that the essential oil could provide a way to control aflatoxin in corn and soybean grains, which are typically used in poultry nutrition. Body weight and FCR improved with 250 mg of oregano essential oil/kg of diet and when combined with vitamin C (200 mg/kg of diet) when compared with those fed a control diet (Ghazi et al., 2015). Furthermore, Vázquez et al. (2015) compared the impact of two levels of addition of Mexican oregano oil (4% thymol + 60% carvacrol or 40% thymol + 20% carvacrol) in broiler feed on water intake, FI, FCR and body weight gain. They found these combinations had more beneficial effects on water intake, FI and FCR than if they were used separately. In Japanese quail, FI intake was clearly increased in the group that received 50 mg/kg oregano oil in feed in comparison with the other groups (0, 100, 200 and 400 mg/kg diet), but FCR was significantly decreased (Badiri, 2016). Using oregano oil in birds' diet increases feed utilisation by the stimulating digestion process. Also, there is some proof that using herbs, spices and different plant extracts is appetising and digestion-stimulating. These facts can explain the increasing FI and improving FCR in Japanese quails (Badiri, 2016).

Some authors tested the effect of thymol and cinnamaldehyde at a level of 100 ppm in the diets on enzyme activities in broiler chickens (Lee et al., 2003, 2004). An increase in lipase by 29% and trypsin activity increased by 18% in the gastrointestinal tract by supplement of thymol in chicken diets. Jang et al. (2007) noticed a significant increase in activities of pancreatic amylase, maltase and trypsin in broiler chickens that received different blends of commercial essential oils.

A trial study in broilers was carried out to determine the effect of oregano essential oil derived from *O. vulgare* L. on growth, feed utilisation efficiency in broiler chickens. Diets with essential oil were formulated to contain 3% less energy (Van Eerden et al., 2012). In that study, the essential oil was used as a commercial product, based on oregano essential oil that consists of main isomers of carvacrol and thymol (these two active substances comprising almost 81% to 82% of the total oil) and more than 30 other substances including p-cymene and g-terpinene. This experiment used essential oil at two levels (0 and 150 mg/kg) and dietary energy at four levels. The level of 150 mg/kg essential oil was used in trial studies. Reduction of the fat content was used to decrease apparent metabolisable energy (AME) content in the diets while maintaining dietary crude protein levels. In the starter period (Days 0–15), all diets had the same energy level. During the grower phase (Days 15–30) and the finisher phase (Days 30–36), the energy treatment was implemented with a reduction of 30, 60 and 90 kcal/kg of diet. The addition of essential oil to the diet resulted in a notably increased body weight and markedly lower feed to gain ratio. However, no major differences were observed in FI. The results showed a 2.4% and 1.1% increase in growth rate and feed efficiency, respectively, during the starter phase, indicating an energy-saving effect in essential oil supplemented groups. As the reduction in dietary apparent metabolic energy (AME) content resulted in an increased feed intake (FI), the energy conversion efficiency (ECE), and weight gainis

TABLE 7 The studies of thymol and carvacrol effects on growth performance in poultry

Studied compound	Effects	Species of poultry	Reference
Oregano EO (natural and synthetic) (200 mg/kg)	Increased ADG, decreased FCR during days 1 to 21	Broiler chickens	Zhang et al. (2021)
Micro-encapsulated blend of EO containing 60% cinnamaldehyde and 30% carvacrol (100, 200 and 400 mg/kg)	Increased FI, BWG	Broiler chickens	Bosetti et al. (2020)
Thymol (300 mg/kg of diet) Carvacrol (300 mg/kg of diet) Eugunol (300 mg/kg of diet) (From 28 to 78 weeks of age)	Improved FI, FCR	Layer chickens	Ghanima et al. (2020)
PFA containing thymol, carvacrol and cinnamic aldehyde (0.5 and 1% of diet)	Improved BW in 35 and 42 days of age	broiler chickens	Reis et al. (2018)
1.33 mmol of thymol/kg feed	No effect on FI and FCRFinal BW increased (at 42 days old)	Broiler chickens	Luna et al. (2018)
Encapsulated mixture of thymol, carvacrol and limonene (100 mg/kg)	Increased overall BWG, no effect on FI, feed efficacy during finisher phase improved	Broiler chickens	Hafez et al. (2016)
EO containing 25% thymol and 25% carvacrol (60, 120 and 240 mg/kg)	0-14 days of age: performance not affected 14-28 days of age: FCR decreased linearly	Broiler chickens	Du et al. (2016)
Carvacrol (300 and 500 mg/kg of diet for 42 days) Thymol (250 mg/kg of diet)	Not improve FI and BWG Increased BWG	Broiler chickens	Shad et al. (2016)
Oregano EO (300 and 600 mg/kg)	Increased BWG, on Day 28: 600 mg/kg of EO decreased FCR	Broiler chickens	Peng et al. (2016)
Thymol (leaves and flowers without stems (1.4% EO, 58% thymol, 0.1%–1% in diet for 35 days)	No clear effect of thymol on performance	Broiler chickens	Haselmeyer et al. (2015)
Supplementary mixture containing 5% carvacrol, 3% cinnamaldehyde and 2% capsicum (100 g/t)	Increased BWG (14.5%)Improved feed efficiency (9.8%)	Broiler chickens	Bravo et al. (2014)
Thymol and carvacrol (200 mg/kg of feed of each compound)	Improved FI, BWG and FCR	Broiler chickens	Hashemipour et al. (2014)
Thymol andcarvacrol (60, 100 and 200 mg/kg of diet)	Decreased FI, with highest BWG and feed efficacy at the high concentration	broiler chickens	Hashemipour et al. (2013)
Thymol and oregano EO (thymol and carvacrol)	Increased ADG and improved FCR	Broiler chickens	Abd EL-Wareth et al. (2012)
200 ppm carvacrol (1 day old for 4 weeks)	Reduced FI and BWG, improved FCR	Female broiler chickens	Jaafari et al. (2012)
5 ppm carvacrol (1 day old for 7 days)	Improved BWG	Broiler chickens	Lillehoj et al. (2011)

(Continues)

TABLE 7 (Continued)

Studied compound	Effects	Species of poultry	Reference
Thymol (250 g/kg) and carvacrol (250 g/kg)	FI, BWG and FCR were not improved with supplementation of thymol and carvacrol (250 and 250 g/kg)	Broiler chickens	Akyurek and Yel (2011)
Rosemary volatile oil (100, 150 and 200 mg/kg)	No effect on FI, but BWG and feed efficacy	Broiler chickens	Yesilbag et al. (2011)
Thymol (0.2, 0.4 and 0.8 g/kg)	No effect on performance and FCR in 28 days old	Broiler chickens	Hoffman-Pennesi and Wu (2010)
Thymol (100 and 200 ppm) for 42 days	Increased FI, BWG and FCR	Broiler chickens	Al-Kassie (2009)
Carboxymethyl cellulose diet with 200-ppm carvacrol	No effect on BWG	Broiler chickens	Lee et al. (2004)
Carboxymethyl cellulose diet with 100-ppm thymol	No effect on performance at 28 days of age	Female broiler chickens	Lee et al. (2003)

Abbreviations: ADG, average daily gain; BW, body weight; BWG, bodyweight gain; EO, essential oil; FCR, feed conversion ratio; PFA, phytogenic feed additive.

calculated. The ECE maintained at more or less the same level at different dietary AME content, while with essential oil supplementation, there was a linear increase in ECE with reducing dietary AME content.

1.8 | Antioxidant and anti-inflammatory actions

Free radicals or reactive oxygen intermediates are generated by cells during normal metabolism. When free radicals such as superoxide radical, hydrogen peroxide and hydroxyl radicals are accumulated excessively, this leads to damage in tissue and privation of many cellular functions. Carvacrol is an antioxidant that protects cells against free radicals. Moreover, antioxidants inhibit prostaglandin synthesis and induct drug-metabolising enzymes in addition to many biological activities as reported by Azirak and Rencuzogullari (2008).

Supplemental oregano by 50 to 100 mg/kg to broiler chick diets exerted an antioxidant effect in the broiler tissues (Botsoglou et al., 2002). Ruberto et al. (2000), Alma et al. (2003) and Luna et al. (2010) reported that the diet supplemented with carvacrol or thymol has similar effectiveness to inhibit the oxidation of lipids than the synthetic antioxidant supplementation such as butylated hydroxytoluene, ascorbic acid and vitamin E and could be considered good natural additives to be applied in animal and poultry industry to improve the performance and health. Animals fed a diet supplemented with carvacrol had greater concentrations of superoxidase and glutathione peroxidase and more levels of poly unsaturated fatty acids in the brain phospholipids than the unsupplemented control (Youdim & Deans, 2000).

In an experiment on broiler chickens, Botsoglou et al. (2002) added oregano in doses of 50 to 100 mg/kg to the diet. They found a strong antioxidant effect in the broiler tissues. At high ambient temperatures and during lipid oxidation, thymol exhibited a strong antioxidant activity than other compounds as reported by Yanishlieva et al. (1999). These authors also reported that thymol excelled carvacrol in antioxidant activity because thymol has a greater stearic hindrance of the phenolic group than carvacrol.

According to Cuppett and Hall (1998) and Brenes and Roura (2010), it has been reported that a wide range of herb extracts have antioxidant properties, especially those products derived from the Labiatae family such as oregano, thyme, basil, mint, rosemary, sage, savory, marjoram, hyssop and lavender due to their phenolic terpenoid compounds, for example, carvacrol, thymol, rosmarinic acid, rosmarol, menthol and eygenol. On the other hand, there is some evidence suggesting that the antioxidant properties of phytobiotics are not only caused by their phenolic substances but also their nonphenolic compounds, such as glycosides (Milos et al., 2000). Cherian et al. (2013) showed the addition of dietary extract of *Artemisia annua* to broiler diet can improve antioxidant activity by reducing thiobarbituric acid reactive substances (TBARS) in breast and thigh meat. They suggested that the reduction in TBARS could be due to the combined antioxidant properties of polyphenolic compounds and vitamin E in *A. annua*. Placha et al. (2014) have demonstrated that supplementing the diet of broiler chickens with thymol can reduce the oxidation of fatty acids indicated by the lower malondialdehyde level in duodenal mucosa. Another recent study by Avila Ramos et al. (2017) showed that diet supplementation for 42 days with an oregano extract (with 31% thymol and 10% carvacrol) at the level of 100 mg per kg, increased 55.2% thymol and 64.8% the carvacrol content in breast meat. These authors analysed thymol and carvacrol by the GS-MS method. This modern and accurate analytical methodology can be used to identify and quantify the traceability of herbal phenolic constituents in meat or other animal tissues. The efficacy of herbal extracts as antioxidant feed additives needs to be evaluated and correlated with their phenolic content.

Antioxidant compounds from the herbal extracts have been ingested by the feed, absorbed in the digestive tract and protected meat lipids from oxidative process and oxidation end products such as aldehydes formation in both breast and thigh meats. Despite the fast metabolism of thymol and carvacrol in the digestive tract, abundant quantities were found in chicken meat (Avila Ramos et al., 2017). The bioavailability mechanism of the herbal phenolic compounds can influence the determination of antioxidant health benefits (Jin et al., 2019).

Oxidative stability of eggs was improved by thymol (Liu et al., 2009). The same effect was also observed with oregano (Radwan Nadia et al., 2008), sage (Lopez-Bote et al., 1998), turmeric (*Curcuma longa;* Radwan Nadia et al., 2008), rosemary (Radwan Nadia et al., 2008), tea catechins (Yilmaz, 2006), mulberry leaf, Japanese honeysuckle and goldthread (Liu et al., 2009).

The effect of oregano (*O. onites*) essential oil rich in carvacrol on the extension of shelf life of packed fresh chicken was investigated. Oregano essential oil extended product shelf life by approximately 2 days (Oral et al., 2009). This has been also confirmed in studies with the Greek oregano oil (Giannenas et al., 2003).

Thymol has been shown to possess anti-inflammatory properties. It suppresses the maturation of dendritic cells and activation of T cell proliferation in vitro (Amirghofran et al., 2016). In the jejuna of broilers, supplementation with thyme powder, which contains the essential oils thymol (50.48%), γ-terpinene (11.03%), p-cymene (9.77%) and carvacrol (4.30%), significantly decreased transcriptional factor nuclear factor kappa B (NFκB). Pro-inflammatory cytokines such as Interleukin-6 (IL-6,) Interferon gamma (IFN-γ) and Tumour Necrosis Factor alpha (TNF-α) were also downregulated in broilers following treatment with thyme powder (Hassan & Awad, 2017). Reduced expression of IL-6 and LPS-induced TNF-α factor was observed in *Eimeria* oocyst-challenged broilers supplemented with essential oils containing 81.89% carvacrol (Lu et al., 2014). This is consistent with previously mentioned results, suggesting that the anti-inflammatory effects of thymol and carvacrol make it suitable for application in animal production.

1.9 | Gut microbiota modulation

Typhlitis associated with intestinal spirochetes of the genus *Brachyspira* is a disease complex mainly seen in laying hens and broiler breeders. Research work by Verlinden et al. (2013) indicated that among other compounds, carvacrol, thymol, linalool, nerolidol, eugenol, piperine, capsaicin and cinnamaldehyde derived from aromatic plants demonstrated the lowest MICs against *Brachyspira intermedia*. When birds infected with *B. intermedia* were given feed supplemented with trans-cinnamaldehyde (500-mg/kg feed), the enumeration of *Brachyspira* was significantly decreased compared to control hens.

Invasion of Madin–Darby bovine kidney epithelial cells by *Eimeria tenella* sporozoites is inhibited in the presence of carvacrol, curcumin or *Echinacea purpurea* extract and enhanced by betaine. Combining carvacrol with purpurea extract inhibited *E. tenella* invasion more effectively than applying the compounds individually, but the further addition of curcumin did not reduce invasion further (Burt et al., 2013). This in vitro assay has been proved to be a fast and inexpensive tool to screen natural substances against coccidian oocysts in order to discover effective anticoccidials and confirm their inhibiting action (Hessenberger et al., 2016)

Michiels et al. (2008) have also indicated that supplementing with 500 ppm carvacrol and thymol reduces the number of intraepithelial lymphocytes and increases villus height/crypt depth in the distal small intestine.

1.10 | Immunomodulatory effect

Immune stimulation by using herbal extracts may reduce the animal's susceptibility to infectious diseases (Dhama et al., 2015). Improving poultry immunity is one of the main goals to prevent infectious diseases (Gholami-Ahangaran et al., 2014). Immunodeficiency could occur by several factors including abuse of antibiotics, vaccination failure or immune-suppressive infectious diseases (Gholami-Ahangaran et al., 2013). To improve the bird's immunity and decrease the susceptibility to infectious diseases, immune stimulators could be used. Acamovic and Brooker (2005) and De Cássia Da Silveira E Sá et al. (2013) reported that herbs rich in flavonoids such as thymol and carvacrol could improve the immune functions through acting as antioxidants and extending the activity of vitamin C. Botsoglou et al. (2002) expected to find an improvement in the immune responses of chicks because of the assured antioxidant, antibacterial and antiviral activities of carvacrol, which had been reported by many researchers. Lillehoj et al. (2011) pointed out that feeding birds with diets containing plant-derived phytobiotics such as carvacrol, thymol, cinnamaldehyde, capsicum and oleoresin significantly improved the immune response in chickens and lowered poultry infectious diseases. Furthermore, Mosleh et al. (2013) observed that using *Z. multiflora* essential oil (that contains thymol) in the chicken diet can stimulate humoral immune responses by increasing antibody titers. The host immune condition plays a major role in resistance to different infections. Phagocyte system, humoral and cellular immune responses of broiler chickens could be enhanced by using essential oils that increase the ability of the defence system to cope with infectious organisms in addition to the immunostimulating activity of polyphenol fraction of thymol and carvacrol (Pérez-Rosés et al., 2015).

Immunised chickens fed the carvacrol/cinnamaldehyde/capsicum-supplemented diet showed increased numbers of macrophages in the intestine, while those given the *Capsicum*/*Curcuma*/oleoresin-supplemented diet had increased numbers of intestinal T cells, compared with untreated controls. While numerous studies have shown disease prevention or immune-enhancing effects of phytochemicals, few have examined the underlying mechanisms that are involved. Some phytochemicals inhibit the innate immune response by targeting pathogen pattern recognition receptors or their downstream-signalling molecules (Furness et al., 2013).

1.11 | Hepatoprotective effect

In a D-galactosamine induced rat model, carvacrol exhibited a hepatoprotective role either in vivo or ex vivo. Aeschbach et al. (1994), Aristatile et al. (2009) and Guimarães et al. (2010) revealed that using carvacrol at the level of 80-mg/kg body weight in rats helped in restoring the concentrations of lipid peroxidation products, lipids content in kidney, liver and blood plasma to its normal values. In addition, enzymic and non-enzymic antioxidants concentrations induced by D-galactosamine were also restored to normal by carvacrol. The aforementioned authors added that the treatment with carvacrol restored and controlled the damage of DNA and the reductions in mitochondrial enzymes induced by D-galactosamine. In fact, the lack of glucose and oxygen needed for the metabolism of the cell could have happened if blood flow to an organ was insufficient or stopped resulting in ischemia. Reperfusion is a term for the restoration of blood flow to the tissue after the elimination of the causative agent for ischemia. As a result of reperfusion, toxic products pass to the circulation system. During liver surgery, renal Ischemia/reperfusion (I/R) injury and liver transplantations, hepatic ischemia is a frequent problem. Canbek et al. (2008) and Aristatile et al. (2009) reported that carvacrol protects the liver during renal I/R injury and hepatic I/R injury through improving liver antioxidant defence and minimising the products of lipid peroxidation.

1.12 | Egg production

Thyme (*T. vulgarus*) as a feed supplement has recently been reported to exert a wide range of beneficial effects on egg production and egg quality (Khan et al., 2012). Bölükbaşi & Erhan (2007) studied the effect of dietary supplementation with thyme on the performance of laying hens and *E. coli* concentrations in their faeces. Thyme addition to basal diet at the level of 0.1%–0.5% have given an improvement in feed conversion and egg production associated with a decline of *E. coli* concentration in faeces. Lohmann Brown hens fed phytogenics consumed less feed and had higher egg production as compared to the control group. Total and average daily FI was lower by 1.8% when supplemented with phytogenics (Nichol & Steiner, 2008). Effect of poly fatty acid on egg quality traits, such as yolk composition, shell thickness or Haugh unit rating, were reported in a few studies only, whereas the majority of reports did not identify substantial effects (Navid et al., 2013). Recent trials carried out with a standardised phytogenic additive (Biomin P.E.P.) confirm the potential of these substances in laying hen nutrition. Especially shell thickness and Haugh unit rating revealed higher values in hens fed phytogenics as the birds grew older. Moreover, eggs were slightly and significantly heavier in these hens as indicated by a higher number (29.5% vs. 23.1%) of heavier eggs, particularly towards the end of the trial.

Ghanima et al. (2020) designed a study for evaluation of thymol effect on egg production and represented that layers that received thymol had the highest egg production at all experimental periods. There were significant differences in egg production during some periods (52–60, 60–68 and 68–76 weeks) among the groups due to the interaction effect. In addition, Bozkurt et al. (2012) revealed that essential oil mixture (including thymol and carvacrol) supplementation to the laying hen diet significantly augmented the egg weight and egg production rate in comparison with the control diet. Egg weight and egg mass were positively linearly affected by essential oils supplementation (Bolukbasi et al., 2010; Olgun, 2016). The addition of an essential oil mixture (36 mg/kg) boosted egg weight in the experiment (Ozek et al., 2011). Supplementation of thymol (250 mg/kg) resulted in improved productive performance of laying hens (Abdel-Wareth et al., 2018). Improvements in egg production may be attributed to increased dietary nutrients digestibility and the digestive capacity that induce the intestinal availability of these nutrients for the benefit of the body (Windisch et al., 2008). Olgun (2016) reported that essential oils might improve the ovary functions and the nutrients digestibility in the intestine and consequently increase egg weight and egg mass in laying hens.

Ramirez et al. (2021) used a powdered product containing 16% essential oil extracted from *L. origanoides* that had guaranteed concentrations of 8% thymol and 4.9% carvacrol per kg of the product as a source of essential oil in 70-week-old Isa Brown laying hens in the production phase. The addition of 150-ppm essential oil to the diet improved the percentage egg production and egg mass, as well as the external and internal quality of the egg, compared with the control layers. In addition, both the 80-ppm essential oil and 150-ppm essential oil treatments improved the yolk colour, shell thickness and shell colour, as well as parameters related to the intestinal morphometry, compared with the control group. The findings of this study indicate that 150-ppm essential oil can improve the performance, egg quality and parameters related to the intestinal morphometry of ISA Brown laying hens. Also, Özek et al. (2011) showed that the addition of a mixture of essential oils based on herbs, organic acids or mixed additives to a corn and soybean diet did not significantly affect egg weight but did tend to increase egg production, compared with the control diet. However, He et al. (2017) demonstrated that the addition of 100-mg/kg essential oil to the feed significantly increased the average egg weight and the feed conversion ratio, compared with the control group in Hy-Line hens. This variation in the effects of essential oil treatment on productive parameters among studies can be attributed to differences in the genetic lines of birds used, the contents of carvacrol and thymol, the subspecies of plant from which the essential oil was extracted, the administration methods and the doses used.

1.13 | Egg composition

A number of authors have presented data on the influence of egg composition by aromatic plants, including parameters such as protein, cholesterol and glucose, but few studies appear to demonstrate any significant effect. Devi et al. (2017) reported a decrease in yolk cholesterol for the supplementation of feed with *pudina* leaf powder. More studies demonstrate an influence of aromatics on serum cholesterol, for example, thyme, *Echinacea* or rosemary (Alagawany et al., 2017). Peppermint or thyme has been shown to have an effect on protein concentration (Abd El-Hack & Alagawany, 2015), but the authors do not relate this to egg composition. Conversely, Vakili and Heravi (2016) reported no significant difference in cholesterol concentration of yolk in eggs from birds fed thymol vs. control birds (208.2 and 227.2 mg/yolk, respectively), yet an effect was seen in serum cholesterol levels (153.3 and 102.5 mg/dl for control and supplemented birds, respectively). Compounds such as thymol and carvacrol influence lipid digestion via stimulation of digestive enzymes and antioxidant activity, thereby suggesting a possible mode of action for aromatic plants on serum and egg lipids such as cholesterol (Hashemipour et al., 2013). Haugh unit, the principal indicator of internal albumen quality, yolk score and yolk percentage all appear to follow a similarly inconsistent pattern to that observed with protein, cholesterol and so forth. Some studies report aromatics such as rosemary or thyme/thymol-increasing Haugh unit (Alagawany et al., 2017), *Echinacea*-increasing yolk colour (Jahanian et al., 2017) or oregano-increasing yolk index (Radwan Nadia et al., 2008). However, other studies report no effect on egg composition with the same herb or others such as eucalyptus and *pudina* (Devi et al., 2017).

1.14 | Egg shell thickness and sensory quality of eggs

The shell weight tends to decrease by an increase in egg size over the production cycle; hence, the shell tends to become thinner leading to more fragile eggs and an increased risk of rejected eggs. In moulted hens (82 to 106 weeks), a commercial oregano product did not significantly influence egg shell thickness nor percentage (Bozkurt et al., 2016). Similarly, neither dried oregano or rosemary had an effect on shell parameters after feeding 28-week-old hens for 90 days (Radwan Nadia et al., 2008), while the use of thyme powder, thymol, cinnamaldehyde, *Echinacea, Eucalyptus* and oregano in hens ranging from 24 to 54 weeks of age were reported to have no effect (Ding et al., 2017; He et al., 2017). However, data are conflicting. Supplementation of layer feed with rosemary or thyme powder (0.9%) fed to hens from 36 to 52 weeks resulted in a significant increase in shell percentage and shell thickness vs. un-supplemented controls (Alagawany et al., 2017). El-Hack and Alagawany (2015) observed that 9-g/kg thyme powder resulted in a 17% increase in shell percentage and a 20% increase in shell thickness vs. controls. While Abdel-Wareth and Lohakare (2014) saw an increase in shell thickness and percentage following the administration of peppermint leaves (20 g/kg) to 64-week-old hens over 12 weeks, Akbari et al., 2016 reported no such effect after feeding peppermint essential oil to 42-week-old hens over an 8-week period, albeit under conditions of cold stress. Fennel extract has also been associated with a reduction in cracked eggs when fed to 36-week-old hens over an 8-week period (Hadavi et al., 2017).

The sensory quality of the egg is important for the sale of the final product and consumer acceptance. Following feed supplementation with essential oils, compounds such as thymol are detectable at low levels in plasma and tissues with absorption rates ranging between 0.7% in plasma and 0.027% in liver and muscle (Haselmeyer et al., 2014). Plant compounds fed to hens are readily detectable in the egg (Plagemann et al., 2011), although absorption rates from feed to egg yolk appear to be low and close to zero in the case of thymol (Krause & Ternes, 2000) while approximately 37% of thymol appears to be absorbed vs. levels detected in excreta (Fernandez et al., 2017). Compounds such as p-cymene-2,3-diol and thymol from thyme can be found in the egg yolk 4 days after feeding to the hen, reaching a plateau from approximately 16 days after the start of feeding (Krause & Ternes, 1999). Similarly, yolk levels of essential oil compounds such as carnosic acid from rosemary rapidly decline following the withdrawal of the corresponding compound from feed, typically to low levels around 10 days after cessation of feeding (Krause & Ternes, 2000). In both studies, p-cymene-2,3-diol, thymol or carnosic acid was undetectable in albumen.

Given the documented absorption of plant compounds in eggs, a number of studies have evaluated the impact of such compounds on egg sensory quality. Overall acceptability of eggs from hens offered a combination of powders of thyme, fennel seeds, garlic and marigold up to 12 g/kg did not differ from birds fed a blank diet (Saki et al., 2014). Interestingly, eggs from hens fed a combination of whole flaxseed (10% of diet), and 2% thyme meal were deemed more acceptable by a sensory panel than flaxseed alone (Tserveni Gousi, 2001). While there is a focus on improving welfare and production parameters using plant-based products, users should be mindful of the potential impact of such compounds on the sensory quality of eggs. The various compounds in aromatic plants and their derivatives vary in their biological activity and more than likely will have different absorption rates in tissues and deposition in eggs.

1.15 | Meat quality

Data on zootechnical performance, animal health and meat quality of broilers fed with thymol and carvacrol have been reported. Galli et al. (2020) investigate the effect of phytogenic compounds containing thymol and carvacrol (150 mg/kg), and the pectoral muscle was analysed for composition and meat quality, as well as for fatty acid profile. The yellow intensity was significantly higher in the phytogenic-received groups than the control groups. Also, cooking weight losses were significantly higher in the control group. The other variables of chemicophysical composition in the meat did not differ between groups. These additives generate improved meat quality by increasing polyunsaturated fatty acids that are beneficial to health and by reducing lipid peroxidation, increasing meat shelf life (Galli et al., 2020).

Consumer attention has been raised within the past decades regarding the quality of meat and its products (Gholami-Ahangaran et al., 2014). The poultry meat content of polyunsaturated fatty acids is high; so it is susceptible to oxidative deterioration, which negatively impacts the meat quality (Rahimi et al., 2012). Broilers fed diet containing 150 ppm of carvacrol through the experimental period (1–42 days of age) caused a reduction in thiobarbituric acid production, which is proof of lipid peroxidation in samples of thigh (stored for 5–10 days; Alagawany et al., 2015). Similar results obtained by Kim et al. (2010) revealed that carvacrol supplementation minimised lipid oxidation and microbial load in chicken patties stored at low temperature (0–3°C), as well as improved shelf life and quality of poultry meat. So using carvacrol as a natural antioxidant could improve the quality of poultry products.

The colouration of fresh chicken meat is considered a significant factor in the moment of consumer acquisition of meat (Kuttappan et al., 2013). These authors found, in a study with chicken breasts with three degrees of white stripping, consumer rejection above 50%. The colour of the meat is indicative of meat quality. It may range from pale red to shades of grey. Meat colour is related to the type of muscle fibres, myoglobin and haemoglobin in the blood. Both iron-associated substances and those reacting with oxygen may alter the colouration of the meat (Petracci et al., 2015). Bosetti et al. (2020) represented that the inclusion of carvacrol in broiler diets did not influence the lightness parameters and the redness of the chicken meat; however, chickens that received 100 and 200-mg/kg essential oils differed from the others, presenting higher yellowness. This higher value observed breast meat may be explained by the greater body development of the chickens; however, this compromises the aesthetic quality of the marketed product (Kuttappan et al., 2013) and may also be related to a myopathy called white striping.

The pH evaluation is used as a parameter of meat quality, with pH 5.8 to 5.9 considered normal at 6 h after the slaughter. In chicken, meat is used to indicate meat quality, and 6-h post-mortem chicken meat should have a pH between 5.8 and 5.9 (Petracci et al., 2015). Bossetti et al. (2020) obtained the pH between 5.84 and 5.98 at 4 h after slaughter, explaining the values slightly above the desired parameters. pH values after 24 h of slaughter above 6.2 can be indicative of dark, firm, dry meat due to the retention of large amounts of water, affecting shelf life. However, values below 5.8 at less than 4 h after slaughter may indicate poor water retention in addition to the soft and pale appearance, suggesting meat called pale, soft, exudative (Guerrero et al., 2013).

Meat quality can be improved by incorporating natural antioxidants into animal diets, adding these compounds onto the meat surface or using active packaging. Among the positive effects of natural antioxidants on meat characteristics are retarding lipid oxidation and microbial growth (Velasco & Williams, 2011).

Botsoglou et al. (2003a) reported that α-tocopheryl acetate supplementation was more effective than dietary incorporation of oregano essential oil to extend lipid stability of chicken meat in frozen storage. Moreover, there is a synergistic effect between dietary essential oil and α-tocopheryl acetate supplementation in retarding lipid oxidation in raw and cooked turkey during refrigeration (Botsoglou et al., 2003b). Lopez-Bote et al. (1998) observed an additional effect on meat cooked at 70°C, which came from broilers fed on diets containing rosemary and sage extracts, during refrigerated storage for up to 4 days. The strongest effect of dietary oregano essential oil supplementation to reduce lipid oxidation has been found at levels of 100 mg/kg feed in chicken meat (Botsoglou et al., 2003a) and 200 mg/kg feed in turkey meat (Botsoglou et al., 2003b).

It is well-documented that the antioxidative activity of a given essential oil might be attributed to its major constituents as well as its interaction with minor constituents present in the oil. However, the antioxidative activity of essential oils can repeatedly be linked to their phenolic content, such as carvacrol, eugenol and thymol content, which act as free radical scavengers and hydrogen donators. Phenolic compounds are considered the most common and often the most effective free radical scavengers. Their hydroxyl groups are usually the site of hydrogen donation (Jayasena & Jo, 2014).

Thyme essential oil also has a great potential to be used in meat products due to its powerful antioxidant properties. Its effectiveness was tested in fresh chicken breast meat together with balm during 3-weeks storage at 4°C (Fratianni et al., 2010). The obtained results showed that 0.5% of thyme essential oil is enough to reduce radical formation, decrease the lipid peroxidation, avoid the deterioration of sarcoplasmic proteins and decrease deterioration reactions and extend the shelf life of the product. The authors attributed this protective activity to the presence of carvacrol in the composition of these essential oils. Besides, the use of lower doses of thyme essential oil in dry fermented poultry meat sausage (0.25%) and fresh chicken sausage (0.125%) also confirmed the decrease of lipid oxidation without affected proteolysis and sensory properties of sausages (El Adab & Hassouna, 2016; Sharma et al., 2017).

Thyme essential oil, in particular, was the most effective out of the two essential oils, with a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical inhibition percentage of 25%–30%. This confirmed that fresh chicken breast meat treated with essential oils showed minimal lipid oxidation and thereby extended shelf life.

Numerous experimental applications of essential oils as antimicrobial agents in meat and meat products are reported. Monoterpene phenols, such as thymol and carvacrol, interact with the cell membrane by hydrogen bonding, rendering the membranes and mitochondria more permeable and disintegrating the outer cell membrane. They can inhibit the growth of *E. coli* O157:H7, *S. aureus*, *S. enterica* serovar Typhimurium, *Pseudomonas fluorescens* and *Brochothrix thermosphacta* (Di Pasqua et al., 2010).

Skandamis et al. (2002) reported that essential oils from clove, oregano, rosemary, thyme and sage have high inhibitory activity, particularly against gram-positive bacteria rather than gram-negative bacteria (Marino et al., 2001). Thyme and balm essential oils reduced the natural microflora counts present in chicken breast meat. This prevents chicken meat spoilage and extends the shelf life of the fresh product when stored at 4°C (Fratianni et al., 2010). Thyme essential oil (0.5%) significantly limited the growth of *E. coli*, whereas balm essential oil (0.5%) effectively inhibited the growth of *Salmonella* spp.; additionally, both balm oil and thyme oil-treated chicken meat had lower total microbial counts than the control samples.

Chicken treated with thyme essential oil has been reported to show remarkable inhibition of lactic acid bacteria (LAB) growth until the end of storage. This agrees well with the findings of Ouattara et al. (1997) and Gutierrez et al. (2009) on the inhibitory action of these oils against *E. coli* and *Salmonella* spp. in food as well as in vitro models. Additionally, Chouliara and Kontominas (2006) observed a small but statistically significant effect of thyme oil (0.1%) on the extension of shelf life of fresh chicken breast meat.

The addition of essential oils to edible films and coatings prevents the growth of pathogenic and spoilage bacteria. It further enhances sensory properties in foods including meat and meat products. Ravishankar et al. (2009) showed that wrapping pieces of chicken breast with apple films containing 0.5% carvacrol or cinnamaldehyde-active ingredients of oregano and cinnamon oils reduced the growth of *E. coli* O157:H7, *S, enterica* and *L. monocytogenes* after storage at 4°C for 72 h and that the bactericidal activity was greater with carvacrol than with cinnamaldehyde. Additionally, a tomato-based film-forming solution with carvacrol (0.75%) was effective against *E. coli* O157:H7. Similarly, W. -X. Du et al. (2012) showed that edible films such as apple and tomato films containing carvacrol or cinnamaldehyde can be used to protect raw chicken pieces against bacterial contamination; this treatment did not change the sensory qualities of the baked-wrapped chicken and contributed to the nutritional and health benefits of the wrapped chicken pieces. Carvacrol (0.5%) is more compatible with tomato wraps, while a similar concentration of cinnamaldehyde is more effective with apple wraps used on baked chicken pieces.

The combined use of thymol (0–300 ppm), carvacrol (0–300 ppm) and storage methods on the quality of nonconventional poultry patties was evaluated by Mastromatteo et al. (2009). Increasing amounts of thymol and carvacrol decreased the final cell load of lactic acid bacteria (LAB) and *Enterobacteriaceae* in patties packaged in air and *Enterobacteriaceae* packed in Modified atmosphere packaging (MAP) (40% CO_2/30% O_2/30% N_2). Additionally, total viable counts were reduced by the combined use of MAP and thymol. Thus, the authors suggested that the combined use of MAP and EOs may be commercially applicable for improving the preservation of meat-based products. Chouliara et al. (2007) reported the effect of a combination of MAP and oregano oil on total viable counts in chicken meat. Oregano oil (0.1%) and MAP (30% CO_2/70% N_2 or 70% CO_2/30% N_2) exhibited an additive preservation effect that extended the product shelf life by 5–6 days.

2 | CONCLUSION

This review highlights the beneficial applications of the dietary addition of thymol and carvacrol as natural growth promoters with useful activities on feed utilisation, immunity, oxidative status, microbial infections, vital organ health and performance. In addition, thymol and carvacrol can improve egg production, egg composition, egg thickness and behaviour in layers. Also, the antioxidant benefits of these essential oils may protect the quality of feed and meat in broilers. Furthermore, thymol and carvacrol have potential antimicrobial effects to contribute to a final reduction of intestinal pathogen pressure through inhibition of adherence to the mucosa. It has a great role in the enhancement of digestive enzyme activity and absorption capacity. Thymol and carvacrol have beneficial effects on normal gut function and the overall performance of poultry (FI, body weight gain, feed conversion ratio, nutrient digestibility). These effects could be due to its pharmacological properties and beneficial health effects, such as antioxidant, antimicrobial, antiviral, anti-inflammatory, antifungal, gut microbiota modulation and growth-promoting properties. The mode of action of thymol and carvacrol like nutritional, pharmacological, health benefits and biological properties may play a crucial role in its beneficial usages in poultry farms by providing further understanding of the health applications and increasing performance parameters in agriculture species. These effects show that thymol and carvacrol can contribute to the improvement of production performance and therefore production efficiency as well. In general, thymol and carvacrol have positive effects, but the knowledge of their use in poultry nutrition is still limited and requires further research.

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AUTHOR CONTRIBUTION

Majid Gholami-Ahangaran designed the study, reviewed the manuscript and edited the final version; Asiye Ahmadi-Dastgerdi contributed to design the study, collected the data and drafted the manuscript; Shahrzad Azizi participated in drafting the manuscript and edited the references; Asal Basiratpour, Maryam Zokaei and Masoud Derakhshan contributed in search, writing, review, collecting data and resources.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS STATEMENT

This review has been prepared based on the principles of ethics in research approved by the Islamic Azad University, Shahrekord Branch (2015-9324).

DATA AVAILABILITY STATEMENT

All data are available and reserved with the corresponding author, and requests will be answered.

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ORCID

Majid Gholami-Ahangaran https://orcid.org/0000-0002-2725-1091
Asiye Ahmadi-Dastgerdi https://orcid.org/0000-0002-3986-1866
Shahrzad Azizi https://orcid.org/0000-0001-8202-9613
Asal Basiratpour https://orcid.org/0000-0001-8421-1494
Maryam Zokaei https://orcid.org/0000-0002-0473-7267
Masoud Derakhshan https://orcid.org/0000-0002-5217-9511

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