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USE OF ESSENTIAL OILS IN BROILER CHICKEN PRODUCTION – A REVIEW*

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Abstract

Biological activity of volatile plant metabolites is the property that can potentially find application in animal nutrition. Nowadays, the use of bioactive compounds is encouraged in many areas of industry and agriculture, since these substances have similar properties as withdrawn antibiotic growth promoters. Meat poultry production is focused on the maximization of performance parameters, namely rapid chicken growth with low feed consumption, and with the optimum health status of the flock. Essential oils can stimulate the growth and functioning of the body, which translates into both chicken's health and enhanced production parameters. The substances are characterized by a range of effects, are easily biodegradable, and do not usually require a waiting period – hence they can be used in breeding broiler chickens. Given the increasing restrictions imposed on poultry production in terms of food safety and ethical aspects of husbandry, it seems appropriate to look for the use of new, natural substances to be applied in animal production. The article presents the characteristics of essential oils in this context, with a particular focus on their antimicrobial and immunostimulatory properties. The paper also describes production applications of essential oils tested in experiments on hybrid Ross 308 and Cobb 500 chickens.

Key words: essential oils, biological activity, natural alternatives, performance

Natural substances are commonly used in the production of pharmaceuticals or cosmetics, as well as in agriculture. In recent years, there has been an increased interest in biologically active plant substances, especially in the European countries, Japan, and the USA (Jafari et al., 2011). Essential oils or volatile oils are aromatic oily liquids extracted by distillation from plant parts, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits and roots. The term 'essential oil' can be regarded as a poorly defined term, a by-product of medieval pharmacy, and for this reason, the term 'volatile oil' has been proposed as an alternative. Nevertheless, the former term 'essential oil' is used more often. Essential oils are characteristic for their strong smell and varied composition. Chemically, essential oils are complex and highly variable mixtures of constituents that belong to two groups: terpenoids (monoterpenes and sesquiterpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxyderiva-

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tive, and so on) and terpenoids (isoprenoids) (Bakkali et al., 2008; Nazarro et al., 2013). They are characterized by two or three major components at fairly high concentrations (20–70%) compared to other components present in trace amounts. This determines their versatile biological activity, which is to a large extent conditioned by the dominant component. Various essential oils have many properties in common, e.g. they can be vaporized with steam, are lipophilic, liquid at 18ºC, optically active, and well soluble in ethanol, propylene glycol, or in lipids (Gopi et al., 2014).

Compounds and aromas of essential oils can be divided into 2 major groups: terpene hydrocarbons and oxygenated compounds. Hydrocarbons are molecules composed of H and C atoms arranged in chains. These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic, or tricyclic), or aromatic. Terpenes are the most common class of chemical compounds found in essential oils. Terpenes are made from isoprene units (several 5-carbon base units, C_5), which are the combinations of 2 isoprene units, called 'terpene units'. Essential oils consist mainly of monoterpenes (C_{10}) and sesquiterpenes (C_{15}), which are hydrocarbons with the general formula $(C_5H_8)_n$. Diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}) exist in essential oils at low concentrations. The oxygenated compounds are the combination of C, H, and O, and there are a variety of compounds found in essential oils. Oxygenated compounds can be derived from terpenes, in which they are termed 'terpenoids'. Some oxygenated compounds prevalent in plant essential oils are shown as follows: phenols (thymol, eugenol, carvacrol, chavicol, etc.); alcohols (borneol, isopulegol, lavanduol, α-terpineol, nerolidol, santalol, α-santalol, etc.); aldehydes (citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde, etc.); ketones (carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone, etc.); esters (bomyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, etc.); oxides: (1,8-cineole, bisabolone oxide, linalool oxide, sclareol oxide, etc.) and so on (Bakkali et al., 2008; Tongnuanchan and Benjakul, 2014). The major components of a number of essential oils are presented in Figure 1.

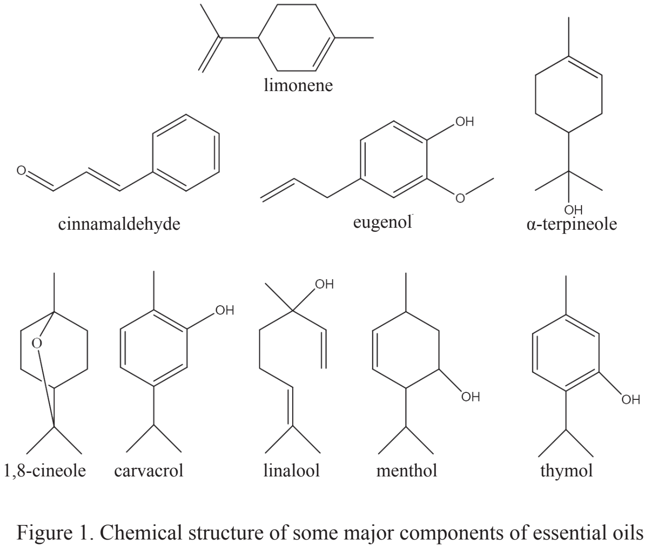

Figure 1. Chemical structure of some major components of essential oils

The literature provides information on numerous characteristics of essential oils, such as antibacterial, antifungal, antiviral, antioxidant, or immunostimulatory properties (Hood et al., 2010; Bharti et al., 2012; Solorzano-Santos and Miranda-Novales, 2012; Alali et al., 2013; Bento et al., 2013; Mahboubi et al., 2013; Krishan and Narang, 2014). Properly selected and composed, the oils can be used as antidiarrheal agents (Gopi et al., 2014). The activity of the essential oils is related to their composition, functional groups, and synergistic interactions between components, for example: the hydroxyl group present in the structure of phenolic compounds confers antimicrobial activity and its relative position is very crucial for the effectiveness of these natural components; this can explain the superior antimicrobial activity of carvacrol, compared to other plant phenolics (Tongnuanchan and Benjakul, 2014).

The aromatic oils used so far in poultry production include oils from oregano (*Origanum vulgare*), mugwort (*Artemisia vulgaris*), onion (*Allium cepa*), mountain savory (*Satureja montana*), Australian tea tree (*Melaleuca alternifolia*), fennel (*Foeniculum vulgare*), turmeric (*Curcuma longa*), lemon balm (*Melissa officinalis*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), cinnamon (*Cinnamomum zeylanicum*), thyme (*Thymus vulgaris*), ginger (*Zingiber officinale*), eucalyptus (*Eucalyptus*), garlic (*Allium sativum*), and clove (*Syzygium aromaticum*) (Bölükbai et al., 2008; de Oliviera et al., 2011; Faramarzi et al., 2013; Akbarian et al., 2014; Drăgan et al., 2014; Feizi et al., 2014; Gopi et al., 2014). Plants potentially used for essential oils may include cedar (*Juniperus virginiana*), lavender (*Lavandula angustifolia*), chamomile (*Matricaria chamomilla*), lemon myrtle (*Backhousia citriodora*) and orange (*Citrus sinensis*) (Muthaiyan et al., 2012; Mahboubi et al., 2013; Prusinowska and Śmigielski, 2014). They are used as additives to feed and drinking water, but also in maintaining facility sanitation, e.g. by fogging or inhalation.

Biological activity of essential oils

Antimicrobial activity

Bacterial diseases still pose a serious problem in the intensive poultry production. The most common bacterial diseases of broiler chickens include salmonellosis, colibacillosis, mycoplasmosis, or infections by *Ornithobacterium* or clostridia. In recent years, a particular concern has been raised by the high incidence of poultry infections by *Salmonella*, *Campylobacter* genus, and *Escherichia coli* (Venkitanarayanan et al., 2013). Problems of large-scale poultry production facilities include diseases caused by microbial complexes, such as *Clostridium – E. coli – Staphylococcus aureus – Eimeria* sp. Analyses of antibacterial properties of essential oils have been carried out by a range of researchers (Ouwehand et al., 2010; Pilau et al., 2011; Solorzano-Santos and Miranda-Novales, 2012; Mahboubi et al., 2013; Nazzaro et al., 2013; Petrova et al., 2013). Several essential oil components exhibit antimicrobial action, some more strongly than others. Phenols, alcohols, ketones and aldehydes are mainly associated with the antibacterial actions, although the exact mechanism of actions has not been fully understood (Nazzaro et al., 2013). The mechanism of action of essential oils depends on their chemical composition, and their antimicrobial activity is not attributable to a unique mechanism but is instead a cascade of reactions involving the entire bacterial cell (Nazzaro et al., 2013). However, it is accepted that the antimicrobial activity depends on the lipophilic character of the components. The components permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane bound electron flow and therewith the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP pool. High concentrations may also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Nazzaro et al., 2013; Gopi et al., 2014).

Essential oils show a particularly strong action against Gram-positive bacteria: *Bacillus cereus*, *Bacillus subtilis*, *Clostridium colinum*, *Clostridium septicum*, *Listeria monocytogenes*, *Staphylococcus aureus*, or *Streptococcus gallolyticus* (Hammer et al., 1999; Si et al., 2009; Owuehand et al., 2010; Jerzsele et al., 2012; Muthayian et al., 2012; Solorzano-Santos and Miranda-Novales, 2012; Mahboubi et al., 2013; Nimbarte and Kulkarni, 2013; Zengin and Baysal, 2014). Essential oils may be an alternative in fighting pathogenic bacteria that developed resistance to many antibiotics (Solorzano-Santos and Miranda-Novales, 2012; de Rapper et al., 2013). It has been demonstrated that some of the oils have strong bactericidal properties against methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) (Chao et al., 2008; Mulyaningsih et al., 2010; Sadlon and Lamson, 2010; Mulyaningsih et al., 2011; Sienkiewicz et al., 2012). According to current knowledge, lavender, thyme, and eucalyptus oil as well as their components enhance their effects in combination with other essential oils or synthetic antibiotics (Sadlon and Lamson, 2010; Bassole and Juliani, 2012; Sienkiewicz, 2012; de Rapper et al., 2013; Zengin and Baysal, 2014).

It has been demonstrated that essential oils also act against Gram-negative bacteria, such as *Campylobacter jejuni*, *Escherichia coli*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Pseudomonas aeruginosa*, *Salmonella enteridis*, or *Klebsiella* sp. (Hammer et al., 1999; Hulankova and Borilova, 2011; Roofchaee et al., 2011; Solorzano-Santos and Miranda-Novales, 2012; Kurekci et al., 2013; Nimbarte et al., 2013; Alali et al., 2013; Cerisuelo et al., 2014; Zengin and Baysal, 2014). Essential oil compounds and their combinations also show antifungal activity (Hammer et al., 1999; Edris, 2007; Hood et al., 2010; Owanagh et al., 2010). Hammer et al. (1999) reported that monoterpenes are effective against yeasts and filamentous fungi. The components of essential oils are also effective against molds of the genus *Aspergillus*, including *A. fumigatus*, which is the most frequent cause of aspergillosis in poultry (Edris, 2007; Esper et al., 2014). The study by Esper et al. (2014) suggests that the oil of oregano (*Origanum vulgare*) may serve as a protective feed supplementation against aflatoxin B_1. Activity of essential oils against pathogenic protozoa is well described (Abbas et al., 2012; Remmal et al., 2013; Gopi et al., 2014). Table 1 lists data on minimum inhibitory concentration (MIC) of some essential oils against microorganisms and Table 2 lists data on MIC of some essential oil components against microorganisms *in vitro.*

Table 1. Minimum inhibitory concentrations (MIC) of some essential oils against microorganisms

Essential oil	Major components (typical composition %)	Species of microorganism	MIC ($\mu g\ ml^{-1}$) or (% $v\ v^{-1}$)*		References
			in vitro	*in vivo*	
1	2	3	4	5	6
Cinnamon (*Cinnamomum zeylanicum*)	cinnamaldehyde (77.1)	*Bacillus cereus*	339	-	Bakkali et al., 2008
	eugenol (7.2)	*Escherichia coli*	200	-	Brenes and Roura, 2010
		Campylobacter jejuni	500	0.25–1.0*	Hyldgaard et al., 2012
		Salmonella sp.	200	-	
Chamomile (*Matricaria chamomilla*)	α-bisabole oxide (30.9)	*Bacillus subtilis*	7.0	-	Bakkali et al., 2008
	α-bisabolol (11.3)	*Enterobacter cloacae*	10.0	-	Soković et al., 2010
	chamazulene (10.9%)	*Escherichia coli*	10.0	-	
		Listeria monocytogenes	9.0	-	
Clove (*Syzygium aromaticum*)	eugenol (76.8)	*Escherichia coli*	0.4–2.5	-	Hammer et al., 1999
	β-caryophyllene	*Staphylococcus aureus*	0.4–2.5	-	Burt, 2004
		Salmonella typhimurium	2–20	-	Bajpai et al., 2012
		Listeria monocytogenes		0.5–1.0*	
Eucalyptus (*Eucalyptus*)	citronelal (72.8)	*Aspergillus niger*	250	-	Brenes and Roura, 2010
	citronellol (14.5)	*Candida albicans*	125	-	Bajpai et al., 2012
		Escherichia coli	250	-	Mahboubi et al., 2013
		Salmonella typhimurium	0.25–250	-	
		Staphylococcus aureus	62.5	-	
Garlic (*Allium sativum*)	diallyl trisulfide(45.9%)	*Candida albicans*	470–970	-	Bajpai et al., 2012
	diallyl disulfide (17.5–35.6)	*Escherichia coli*	15–150	-	Hyldgaard et al., 2012
		Salmonella enteritidis	80–310	-	
		Staphylococcus aureus	12	-	
Ginger (*Zingiber officinale*)	camphene (14.1)	*Salmonella typhimurium*	2.0	-	Brenes and Roura, 2010
	β-bisabolene (22.1)				Bajpai et al., 2012
Oregano (*Origanum vulgare*)	carvacrol (64.5–69,5)	*Aspergillus flavus*	10.0	-	Hammer et al., 1999
	cymene (10,6–10.9)	*Bacillus subtilis*	0.125	-	Soković et al., 2010
	thymol (4,1,)	*Candida albicans*	10–20	-	Hulánková and Bořilová, 2011
		Enterobacter cloacae	0.5	0,03–2.0	Roofchaee et al., 2011
		Escherichia coli	0.5–1.2	1.0–2.1*	Bajpai et al., 2012
		Listeria monocytogenes	0.2–2.36	1.0*	Hyldgaard et al., 2012
		Salmonella enteritidis	0.5	0.6–0.9*	Esper et al., 2014
		Salmonella typhimurium	0.1–3.1	0.03*	Tongnuanchan and Benjakul,
		Staphylococcus aureus	0.25–3.2	0.02*	2014

Table 1 – contd.

1	2	3	4	5	6
Peppermint (*Mentha piperita*)	menthol (37.4)	*Enterobacter cloacae*	3.0	-	Soković et al., 2010
	menthon (12.7)	*Escherichia coli*	2.5	-	Bajpai et al., 2012
	menthofuran (6.8)	*Listeria monocytogenes*	2.5	0.5*	Hyldgaard et al., 2012
		Salmonella enteritidis	3.0	0.5–2.0*	
		Pseudomonas aeruginosa	3.0	-	
		Salmonella typhimurium	1–2.5	-	
		Staphylococcus aureus	2.5–790	-	
Rosemary (*Rosmarinus officinalis*)	1,8-cineole (43.6-50,0)	*Bacillus cereus*	0.2	-	Hammer et al., 1999
	α-pinene (7.4-9,9)	*Candida albicans*	10	-	Bajpai et al., 2012
	camphor (12.3)	*Escherichia coli*	4.5–40; 0.25*	-	Hyldgaard et al., 2012
		Listeria monocytogenes	0.2; 0.12*	1.0–5.0*	Mathlouthi et al., 2012
		Salmonella typhimurium	2–20; 0.25*	-	El-Shenawy et al., 2015
		Staphylococcus aureus	0.4–20; 0.12*	-	
Sage (*Salvia officinalis*)	1,8-cineole (8.4)	*Bacillus cereus*	5.0	0.2–0.5	Hammer et al., 1999
	α-thujone (31.8)	*Bacillus subtilis*	5.0	-	Soković et al., 2010
	β-thujone (33.2)	*Escherichia coli*	3.5–5	-	Brenes and Roura, 2010
		Listeria monocytogenes	7.0	-	Bajpai et al., 2012
		Salmonella typhimurium	6.0–20	0.5–3.0*	
		Staphylococcus aureus	0.7–10	-	
Savory (*Satureja montana*)	carvacrol (37.7)	*Clostridium perfringens*	1.6	1.56*	Brenes and Roura, 2010
	p-cymene (25.9)	*Escherichia coli*	40	-	de Oliviera et al., 2011
		Staphylococcus aureus	5	-	Hyldgaard et al., 2012
Tea tree (*Melalecua alternifolia*)	terpinen-4-ol (40.1)	*Escherichia coli*	0.15*	0.15*	Hood et al., 2010
	γ-terpinene (23.1)	*Salmonella typhimurium*	0.5	0.07*	Bajpai et al., 2012
	α-terpinene (10.4)	*Salmonella gallinarum*	0.07*	0.07*	Nimbarte et al., 2013
		Staphylococcus aureus	0.07*	-	
Thyme (*Thymus vulgaris*)	thymol (48.9)	*Bacillus subtilis*	0.5	-	Hammer et al., 1999
	p-cymene (19.0)	*Candida albicans*	0.03*; 1.0	-	Soković et al., 2010
		Eimeria	0.31*	-	Abbas et al., 2012;
		Enterobacter cloacae	1.0	-	Bajpai et al., 2012
		Enterobacter faecalis	0.5–1.0	-	Hyldgaard et al., 2012
		Escherichia coli	0.25–2; 0.06*	-	Sienkiewicz et al., 2012
		Listeria monocytogenes	0.3–1.0; 0.06*	0.1–1.5*	Nimbarte and Kulkarni, 2013
		Pseudomonas aeruginosa	1.0; 0.06*	-	El-Shenawy et al., 2015
		Salmonella gallinarum	1.0	-	
		Staphylococcus aureus	0.2–5; 0.03–0.06*	-	

Table 2. Minimum inhibitory concentrations (MIC) of some essential oil components against microorganisms *in vitro*

Compounds	Microorganisms	MIC (μg ml^{-1}) or (% v v^{-1})*	References
Carvacrol	*Bacillus subtilis*	0.125	Soković et al., 2010
	Candida albicans	113.0–200.0	Bajpai et al., 2012
	Enterobacter cloacae	0.5	Krishan and Narang, 2014
	Escherichia coli	0.5–225.0	
	Mycobacterium avium	72.0	
	Pseudomonas aeruginosa	1.0	
	Salmonella typhimurium	0.25–0.5	
	Staphylococcus aureus	0.25–450.0	
1,8-cineole	*Bacillus subtilis*	4.0	Soković et al., 2010
	Enterobacter cloacae	6.0	
	Escherichia coli	6.0	
	Listeria monocytogenes	5.0	
	Staphylococcus aureus	5.0	
Cinnamaldehyde	*Candida albicans*	200.0	Bajpai et al., 2012
	Escherichia coli	396.0	Krishan and Narang, 2014
	Salmonella sp.	500	
Eugenol	*Salmonella typhimurium*	0.5–16	Bajpai et al., 2012
Linalol	*Bacillus subtilis*	4.0	Soković et al., 2010
	Enterobacter cloacae	6.0	
	Escherichia coli	6.0	
	Listeria monocytogenes	5.0	
	Pseudomonas aeruginosa	9.0	
Menthol	*Aspergillus niger*	125.0	Soković et al., 2010
	Bacillus cereus	250.0	Mahboubi et al., 2013
	Bacillus subtilis	0.5	
	Candida albicans	125.0	
	Enterobacter cloacae	2.0	
	Escherichia coli	1.0–250.0	
	Pseudomonas aeruginosa	3.0	
	Staphylococcus aureus	1.0–125.0	
Terpinen-4-ol	*Campylobacter jejuni*	0.05*	Kurekci et al., 2013
Thymol	*Bacillus subtilis*	0.25	Soković et al., 2010
	Enterobacter cloacae	1.0	Bajpai et al., 2012
	Escherichia coli	1.0–450.0	Krishan and Narang, 2014
	Pseudomonas aeruginosa	1.5	
	Salmonella typhimurium	0.05–56.0	
	Staphylococcus aureus	0.25–225.0	

Eimeria-caused coccidiosis is the most common parasitic disease in broiler chickens. It is particularly destructive under intensive husbandry on litter. Which particular species of coccidia may occur on the farm depends mainly on coccidiostats that are added to feed. The most recent studies show that mugwort, fennel, or oregano essential oils, as well as their components, including eugenol, isopulegol, carvacrol, carvone, and thymol, can be used in the prevention of coccidiosis in poultry (Abbas et al., 2012; Remmal et al., 2013; Drăgan et al., 2014; Murakami et al., 2014).

Antiviral activity of essential oils is poorly understoood. There are some reports on the activity of some oils on viruses; its mechanisms, however, have not been fully described. Saderi and Abbasi (2011) observed that thyme oil is potentially effective against adenoviruses. Oregano oil and one of its components, carvacrol, have been demonstrated to be effective against entero- and rotaviruses (Pilau et al., 2011; Sánchez et al., 2015).

Antioxidant activity of essential oils

Another interesting issue concerns the antioxidant properties of essential oils. Applied in poultry production, they can reduce lipid peroxidation in the muscles of meat chickens. Thigh muscles are susceptible to oxidation due to a higher content of polyunsaturated fatty acids, oxidation of which produces peroxides, lipids, oxysterols, or malondialdehyde (Tongnuanchan and Benjakul, 2014). Literature data suggest that essential oils are effective in preventing lipid oxidation, similar to α-tocopherol or a mixture of synthetic compounds (BHT/BHA). High efficacy in inhibiting oxidation of fatty emulsions was demonstrated for thyme (88.0%), rosemary (78.8%), sage (73.9%), and lavender (72.5%) oils (M'Hir et al., 2012; Amorati et al., 2013). The use of antioxidative compounds in animal feeds may be a way of controlling and reducing oxidative rancidity in meat (Hashemipour et al., 2013). Antioxidants present in the feed are absorbed in the intestine, so that they can perform their functions at the body level. It has been found that the use of synthetic antioxidants, such as butylated hydroxytoluene (BHT) and α-tocopherol, reduces the rate of lipid oxidation in poultry meat during both cooling and freezer storage (Tongnuanchan and Benjakul, 2014). The antioxidative status of chicken meat can also be enhanced using natural antioxidants, such as essential oils or their ingredients. Their influence on the oxidative stability of muscles was studied in the meat of chickens fed feeds supplemented with thymol, carvacrol, oregano or rosemary oil (Yesilbag et al., 2011; Hashemipour et al., 2013). The studies have shown that chickens fed with the addition of natural vitamin E and rosemary oil had a significantly lower, as compared with the control, values of malondialdehyde concentration in the pectoral muscles (Yesilbag et al., 2011). Hashemipour et al. (2013) found that the addition of thymol and carvacrol in an amount of 200 mg kg^{-1} feed is characterized by a strong antioxidant effect towards lipids in the femoral muscles of chickens (low concentration of malondialdehyde, increased content of polyunsaturated fatty acids). The study by Akbarian et al. (2014) revealed that adding turmeric and oregano oils to a diet significantly reduced the level of malondialdehyde in chicken muscles. On the other hand, an increased concentration of glutathione peroxidase (GSH-Px) and a reduced level of malondialdehyde were measured in experiments on the use of cinnamon oil in feeding broiler chickens (Cifici et al., 2010).

Changes in the activity of some antioxidant enzymes (e.g. glutathione peroxidase) allow determination of the effect of stress factors (such as high temperature, noise, transportation) on the oxidative balance of the bird's body (Vosmerova et al., 2010). Evaluation of such parameters is more and more commonly applied in testing new feed additives, such as natural antioxidants, or checking for toxic agents in a chicken diet (Eraslan et al., 2005; Akbarian et al., 2014). The study by Akbarian et al. (2014) focused on the effect of turmeric oil on the biochemical blood profile, antioxidant enzymes, and the concentration of antibodies in chickens under elevated temperature. The results suggest that turmeric oil supplementation in the amount of 400 mg kg^{-1} feed may alleviate the stress resulting from heat. The authors observed an increase in glutathione peroxidase activity in erythrocytes as well as an increased plasma concentration of the growth hormone. Positive effects of feed supplementation in broiler chickens exposed to heat stress has been confirmed by other authors (Parvar et al., 2013; Gopi et al., 2014).

The study by Rimini et al. (2014) suggests that essential oils, such as those extracted from oranges or thyme, may also be used as natural preservatives of chicken meat in curing. The process prevents lipid oxidation in meat without altering its quality, including pH, flavor, or color.

Immunostimulative and anti-inflammatory effects

Some essential oils positively influence the avian immune system, since they promote production of immunoglobulins, enhance lymphocytic activity, and boost interferon-γ release (Awaad et al., 2010; Faramarzi et al., 2013; Gopi et al., 2014; Krishan and Narang, 2014). Supplementing diets with essential oils containing herbal mixtures positively influenced the activity of the intestinal lymphatic system. The authors observed a reduced number of intraepithelial cells in the small intestine, which suggests possible relaxation of the strain resulting from the gastrointestinal defensive response. Placha et al. (2014) found that the addition of 0.5 g of thyme oil per kg of feed significantly increased IgA levels. Awaad et al. (2010) carried out an experiment on birds vaccinated with the inactivated H5N2 avian influenza vaccine. The experiment revealed that adding eucalyptus and peppermint essential oils to water in the amount of 0.25 ml L^{-1} results in an enhanced both cell-mediated and humoral immune response. Saleh et al. (2014), who applied thyme and ginger oils in the quantity, respectively, 100 and 200 mg kg^{-1} feed, observed an improvement in the chicken immunological blood profile through an increased antibody production. Essential oils are also used as immunomodulators during periods when birds are exposed to stress, acting protectively and regeneratively. Moreover, the oils alleviate the stress caused by vaccination (Barbour et al., 2011; Faramarzi et al., 2013; Gopi et al., 2014). The study by Kongkathip et al. (2010) confirmed the antiviral activity of turmeric essential oil. In recent years studies have been carried out on the use of essential oils in conjunction with vaccination programs, including those against infectious bronchitis (IB), Newcastle disease, and Gumboro disease. The results of the experiments show that essential oils promote the production of antibodies, thus enhancing the efficacy of vaccination (Awaad et al., 2010; Barbour et al., 2010; Barbour et al., 2011; Faramarzi et al., 2013).

Essential oils contain compounds that are known to possess strong anti-inflammatory properties, mainly terpenoids and flavonoids, which suppress the metabolism of inflammatory prostaglandins (Krishan and Narang, 2014). Also other compounds found in essential oils have anti-inflammatory, pain-relieving, or edema-reducing properties, for example linalool from lavender oil, or 1,8-cineole, the main component of eucalyptus oil (Peana et al., 2003).

Effect on the digestive and respiratory systems

Essential oils have a positive effect on the avian digestive system, since they help to restore the microbiota balance and increase nutrient absorption, which may chiefly be attributed to terpenoid compounds (Mountzouris et al., 2011; Barbour et al., 2013). It is very important in terms of feed conversion (Mountzouris et al., 2011; Mathlouthi et al., 2012). These compounds also boost the production of digestive enzymes, resulting in better digestion and absorption of nutrients. Essential oils also help to improve protein digestion by increasing the secretion of hydrochloric acid and pepsin (Gopi et al., 2014). In addition, the substances contained in essential oils affect the taste and smell of the feed, which stimulates the secretion of saliva and gastric juices. However, some of the oils may be irritant to the mucous lining of the gut, resulting in inflammation. It is important, therefore, to appropriately select, compose, and dose essential oil supplementation.

Essential oils that influence the respiratory system include oils of peppermint and eucalyptus, which thin the mucus and facilitate its removal from the airways. These oils contain, among others, eucalyptol and menthol, which have antispasmodic and expectorant effects. As a result, the airways are cleared and breathing during inflammation becomes easier (Durmic and Blache, 2012). It is also important in the production houses, especially in summer, when high temperatures and low humidity will result in an increase in air dust. Under such conditions, respiratory tract disorders in broiler chickens, including the deposition of particulates, become more common and more severe. Medications intended as a support in the treatment of respiratory disorders contain thyme oil and its main components, thymol and carvacrol. These substances are antispasmodic to smooth muscles and stimulate the respiratory system. An additional advantage is their expectorant and spasmolytic character (Edris, 2007). Another positive effect of the terpenoid compounds used in commercial preparations for poultry is that they disinfect the bronchi, preventing respiratory infections (Awaad et al., 2010; Barbour et al., 2011; Mahboubi et al., 2013).

Application of the essential oils in broiler chicken production

The replacement of antibiotic performance enhancers with other safe and natural substances is an important objective for the poultry industry. Considering the versatility of essential oils, they can be used as growth promoters in poultry production. There are some promising results concerning the use of essential oils and other natural products as performance enhancers. Typical performance parameters for poultry rearing are body weight, growth, feed intake and feed conversion ratio. A range of authors report the positive impact of essential oils on poultry production performance (Calislar et al., 2009; Al-Kassie et al., 2010; Roofchaee et al., 2011; Erhan et al., 2012; Hong et al., 2012; Vukić-Vranješ et al., 2013; Aguilar et al., 2014; Azadegam Mehr et al., 2014; Karadas et al., 2014; Saleh et al., 2014; Zeng et al., 2015). Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) are presented in Table 3. Studies show different effects of supplementation with essential oils.

Table 3. Effect of essential oils on the performance of broiler chicken

Dose (mg kg^{-1}) or (% w w^{-1})*	Essential oils or their components	Treatment effect (% difference from untreated control)			References
		ADG[1]	ADFI[2]	FCR[3]	
0.5*	peppermint	+18.6	–9.2	–23.5	Al-Kassie, 2010
75	ginger	+7	+6	0	Zeng et al., 2015
100	basil, caraway, laurel, lemon, oregano, sage, tea, thyme	+7	0	–6	Khattak et al., 2014
100	thyme	+20.6	+26.5	+13.2	Saleh et al., 2014
125	oregano	+5	–2	–6	Hong et al., 2012
150	rosewood	+2	+1	–1	Aguilar et al., 2014
150	ginger	+7	+6	0	Zeng et al., 2015
150	oregano	–2	–6	–4	Brenes and Roura, 2010
150	rosemary	0	–1	–1	Brenes and Roura, 2010
150	carvacrol, thymol, 1,8-cineol	+7	-	–3	Alali et al., 2013
150/150	oregano/garlic	–4	–5	–2	Kirkpinar et al., 2011
200	carvacrol	+2	+2	–1	Brenes and Roura, 2010
200	thymol	–5	–3	–3	Brenes and Roura, 2010
200	basil, caraway, laurel, lemon, oregano, sage, tea, thyme	+7	0	–7	Khattak et al., 2014
250	oregano	+3	+4	0	Zeng et al., 2015
300	basil, caraway, laurel, lemon, oregano, sage, tea, thyme	+6	–2	–6	Khattak et al., 2014
300	oregano	–7	–4	+2	Kirkpinar et al., 2011
300	oregano	–3	+1	–2	Brenes and Roura, 2010
300	rosemary	–2	+1	–4	Brenes and Roura, 2010
300	garlic	–3	–4	0	Kirkpinar et al., 2011
300	ginger	+16	+0.5	–13.6	Saleh et al., 2014
300	oregano	+3	+2	–1	Roofchaee et al., 2011
300	thyme	+8	+0.3	–7.2	Saleh et al., 2014
450	rosewood	+1	–1	–2	Aguilar et al., 2014
500	oregano	+3	–3	+8	Zeng et al., 2015
500	basil, caraway, laurel, lemon, oregano, sage, tea, thyme	+7	–2	–8	Khattak et al., 2014
600	oregano	+5	0	–5	Roofchaee et al., 2011
600	rosewood	+1	+2	0	Aguilar et al., 2014
1,000	mint	–1	0	+1.5	Demir et al., 2008
1,000	thyme	–4	-3	0	Zeng et al., 2015
1,000	thyme	–2	0	+2.5	Demir et al., 2008
1,200	oregano	+3	–2	–4	Roofchaee et al., 2011

[1] ADG – average daily gain; [2] ADFI – average daily feed intake; [3] FCR – feed conversion ratio.

It appears that the application of essential oils as growth stimulator substitutes in broiler diets does not always improve production performance, and sometimes even makes it worse (Demir et al., 2008; Ocak et al., 2008; Brenes and Roura, 2010; Kirkpinar et al., 2011; Saleh et al., 2014; Zeng et al., 2015). This is probably due to a wrong oil concentration or too short a time of application. The differences in the reported results may be due to involving weak chicks, breaches of biosafety rules, or the impact of environmental factors, such as bedding, lighting, equipment, presence of rodents etc. This discrepancy may have also been a result of dietary flaws during the experiments, e.g. unbalanced feed, or contaminated feed or water.

Essential oils also find application in drinking water (Manosub, 2011; Alali et al., 2013; Feizi et al., 2014; Khosravinia, 2015; Galal et al., 2016). Average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and mortality rate (MR) are presented in Table 4. Studies show different effects of supplementation with essential oils.

Alali et al. (2013) studied the effects of a mixture of oils (eucalyptus, thyme, lemon) added to drinking water on the production parameters, mortality, water intake, and *Salmonella enterica* colonization in broiler chickens. A considerable improvement in feed conversion and weight gains was observed after the application of 0.025 and 0.0125% of oil mixture. On the other hand, the concentration of 0.05% of oils added to water reduced *Salmonella* counts in fecal smears. In addition, the study by Khosravinia (2015) has shown that not only does an addition of savory oil to drinking water improve production performance, but also reduces external damage to the carcass, which is associated with a reduction in litter moisture and the antibacterial properties of the oil.

Table 4. Effect of essential oils in drinking water on the performance of broiler chicken

Dose (%v v^{-1}) or (ml L^{-1})	Essential oils or their components	Treatment effect, % difference from untreated control				References
		ADG[1]	ADFI[2]	FCR[3]	MR[4]	
0.005	oregano	+1.7	–0.1	–2.6	-	Galal et al., 2016
0.01	oregano	+1.8	+1.5	0	-	Galal et al., 2016
0.05	essential oil blend: eucalyptus, thyme, lemon	+14.8	-	–7.3	–26	Alali et al., 2013
0.1	oregano	+0.1	+0.5	–0.6	-	Manosub, 2011
0.2*	oregano	+0.4	+2.0	–11.0	-	Manosub, 2011
0.4*	savory	+4.7	–1	–1.1	–30	Khosravinia, 2015
0.5*	thyme	+1	–6.6	–7.6	–20	Feizi et al., 2014
1.0*	thyme	+1.8	–7.9	–9.5	–33	Feizi et al., 2014

[1] ADG – average daily gain; [2] ADFI – average daily feed intake; [3] FCR – feed conversion ratio; [4] MR – mortality rate.

Unfortunately, only few studies have been performed to observe the effect of essential oils on microbial numbers in broiler chicken. *In vivo* studies found inhibiting effects against pathogens such as *Clostridium*, *Salmonella* spp., *E. coli* or *Coccidia* (Table 5). The controlled pathogen load also contributed to healthy microbial metabolites, improved intestinal integrity and protection against enteric disease (Kirkpinar et al., 2011; Roofchaee et al., 2011; Erhan et al., 2012; Hong et al., 2012; Zeng et al., 2015).

Table 5. Effects of essential oils and their components on the microflora in broiler chicken

Essential oils	Dose (mg kg^{-1}) or (% v v^{-1})*	Segment of intestine	Microflora	Effect on the microflora (%)	References
Garlic (*Allium sativum*)	0.1*	ileum	*Escherichia coli* *Lactobacillus* spp.	–26.8 + 21	Rahimi et al., 2011
Thyme (*Thymus vulgaris*)	0.1*	ileum	*Escherichia coli* *Lactobacillus* spp.	–38 +31.6	Rahimi et al., 2011
Mint (*Mentha pulegium*)	0.25*	jejunum	*Escherichia coli* *Lactobacillus* spp.	–34.9 + 57.4	Erhan et al., 2012
Rosemary (*Rosmarinus officinalis*)	1.0	cecum	*Escherichia coli* *Salmonella* spp. *Stephylococci* *Coccidia*	–32.7 –100 –14.6 –100	Tollba, 2010
Blend: oregano, anise, citrus (Biomin®)	125	ileum	Coliforms *Salmonella* *Enterococci* *Lactobacillus* spp.	–0.9 –1.1 –2.6 –2.3	Hong et al., 2012
Garlic (*Allium sativum*)	300	ileum	Coliforms *Clostridium* spp. *Streptococcus* *Lactobacillus* spp.	–10.7 –1.2 –3.2 + 7.0	Kirkpinar et al., 2011
Oregano *(Origanum vulgare)*	300	ileum	Coliforms *Clostridium* spp. *Streptococcus* *Lactobacillus* spp.	–22.7 –13.5 –4.0 +9.2	Kirkpinar et al., 2011
Myrtle (*Myrtus communis*)	300	cecum	*Escherichia coli* *Lactobacillus* spp.	–38.5 +29.6	Ghazanfari et al., 2014
Oregano *(Origanum vulgare)*	300	cecum	*Escherichia coli* *Lactobacillus* spp.	–11.0 –2.0	Roofchaee et al., 2011
Oregano *(Origanum vulgare)*	600	cecum	*Escherichia coli* *Lactobacillus* spp.	–10.3 0	Roofchaee et al., 2011
Blend: oregano, anise, citrus (Biomin®)	1000	cecum	*Escherichia coli* *Enterobacteriaceae* *Enterococcus* *Staphylococcus* *Clostridium* *Lactobacillus* spp.	–7.7 –16.7 –16.9 –4.4 –1.6 –3.9	Vukić-Vranješet al., 2013

Essential oils can improve the hygiene level in the production facilities. According to Witkowska and Sowińska (2013), oil of peppermint and thyme can be used for fogging the production houses. Both oils were effective, although thyme oil had a stronger effect against *Coli* bacteria, whereas peppermint oil had a stronger inhibitory effect on the proliferation of staphylococci. The data resulting from the experiment reveals that the on-farm application of both oils is potentially beneficial; the information on their dosage, however, is missing from the report.

Other applications of essential oils consist in their administration with vitamin E at the final stage of chicken growth. As a result of this treatment, the oxidative stability of meat and its products increases. Both color fastness and water-holding capacity improve. The meat also smells and tastes better (Tongnuanchan et al., 2014). The most recent findings show that essential oils can be applied as natural antibacterial agents in fresh meat preservation. For example, the application of 2% rosemary oil extended the shelf life of chicken breast meat (Ramos et al., 2011; Petrova et al., 2013; Tongnuanchan et al., 2014). Essential oils of chili peppers and onion accelerate the blood circulation, allowing faster clearance of toxins from the broiler's body. One must bear in mind, however, that onion or garlic oils negatively affect the smell and flavor of meat, so a longer waiting period should be allowed for.

Summary

Essential oils are the subject of both *in vitro* and on-farm (*in vivo*) experiments on poultry. There is strong evidence that essential oils have a positive effect on the production performance of broiler chickens, which is reflected in reduced feed intake, increased body weight gains, and better immunity and health. Therefore, new preparations for poultry are constantly being offered, which contain essential oils having strong bacteriostatic properties. Undoubtedly, a great advantage of the essential oils is the fact that no bacterial antibiotic resistance that might be developed as a response to their constituents has ever been reported. Essential oils are used in quite a wide range of dosages, and an additional advantage is that they can be administered besides vaccination. In contrast to chemotherapeutic agents, phytobiotics do not burden the bird's organism and do not require a waiting period before slaughter, which guarantees food safety.

In conclusion, essential oils are the bioactive substances that have many potential applications, though the literature often lacks important information on the details of their dosage. Further research is needed in order to fully evaluate the potential of essential oils in animal production. The results published so far, which prove the beneficial effects of essential oils, should be supported by additional analysis that would preclude any methodological flaws, such as an insufficient number of replications, varying environmental conditions, or too short a time of application.

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