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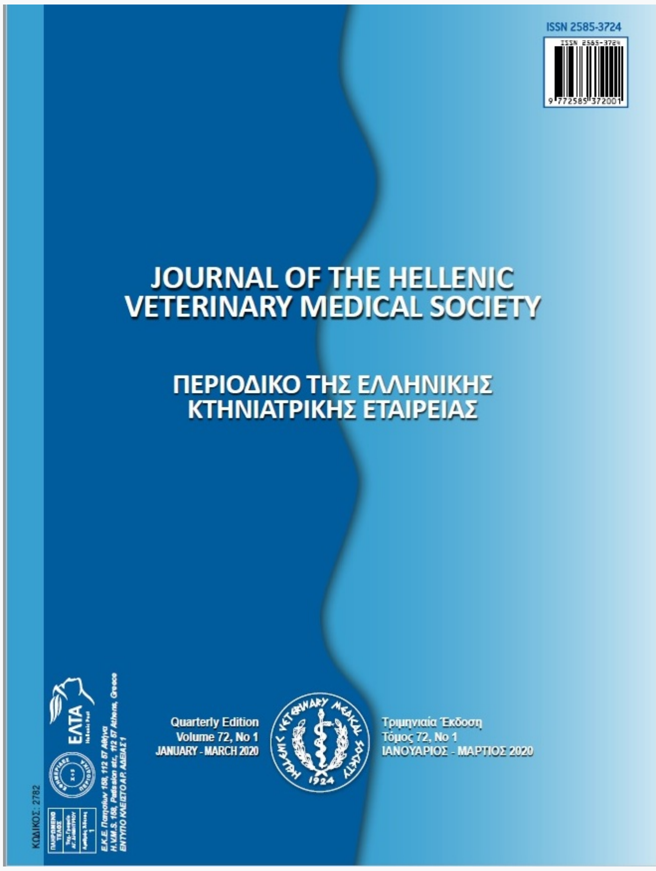

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Review article
Ανασκόπηση

Essential oils as alternatives to chemical feed additives for maximizing livestock production

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ABSTRACT: This review is aimed at providing basic and current knowledge about possible mechanisms and nutritional applications of essential oils (EOs) for food animals. Public concern on the excessive use of antibiotics in livestock production has started extensive research to find safe and efficient options. EOs extracted from aromatic plants are known to have a range of biologically active properties that can be applied to modern animal production. Primarily, EOs possess anti-inflammatory, anti-microbial, and digestion enhancing effects as they improve digestive enzymes, improve feed conversion ratio, modulate ruminal fermentation, add antioxidant properties, and underpin animal immunity. The dietary supplementation of EOs demonstrated as a simple and proficient approach to enhance the performance of livestock. However, mechanisms involved in enhancing animal performance, modulating ruminal fermentation, and microflora are still unclear. Moreover, limited information is available regarding interactions among feed, EOs, and gut ecosystem of animals. EOs could be used as nutraceuticals with possible commercial applications in modern animal nutrition such as antimicrobials, antioxidants, growth promoters, and immunomodulators, alternatives to chemical feed additives. This knowledge encourages further investigations about EOs to realize their full potential and build up their standard use in livestock production.

Keywords: Essential oils; poultry; ruminants; antibiotic resistance; pigs

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INTRODUCTION

For a long time, dietary supplementation of chemical feed additives started to increase animal growth, performance, and efficiency. Antibiotics supplemented in the animal diet at a sub-therapeutic level is intensifying livestock production, reducing morbidity and mortality but also associated with the development of antimicrobial resistance that may present a risk to human health. Correspondingly, natural feed additives extracted from herbs, plants, and spices such as EOs have been evaluated and considered as a substitute to chemical feed additives in livestock production for improving animal production and health. EOs are complex mixture of different components, hence chances of development of resistance in microbes are less as compared to the single synthetic compound. In terms of biological activity and effects, each constituent of EO possesses its characteristic properties. EOs hold the potential of possible therapeutic exploitation in different ways in animal production. They represent a wide range of biologically active compounds like phenolic and terpenoids which possess a variety of functions with health-related benefits and nutrigenomics implications on the development of the gut and immunity (Christaki et al., 2020). In terms of ruminant nutrition, EOs enhance animal performance, manipulate rumen fermentation such as increase protein metabolism, reduce ammonia and methane production, improve volatile fatty acids (VFA) proportions and target some ruminal microorganisms like methane-producing archaea and hyper-ammonia producing (HAP) bacteria (Campolina et al., 2020; Hart et al., 2019; Silva et al., 2020; Tapki et al., 2020; Zhou et al., 2020). They also possess remarkable effects on monogastric animals like improve digestive secretions, body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), meat, and egg qualities (Ismail, El-Gogary, and El-Morsy, 2019; Lee et al., 2020; Masood et al., 2020; Sariözkan et al., 2020; Yalçin et al., 2020). They also exhibit antioxidant properties, stimulate blood circulation, reduce the pathogens count, enhance digestion and nutrient uptake, and relieve the animals from disease and environmental stress. However, due to the intricacy of the animal body systems and EOs composition, the dosage level and effects of EOs on different animal species and systems seem to be difficult to predict (Puvača et al., 2020). To date, only a few studies evaluated EOs with a known chemical composition in modulating their effects and function, while the mode of actions of underlying these functions has not been completely clarified yet (Simitzis, 2017; Zeng et al., 2015). Moreover, the chemical composition of EOs depends on species, topographical location, harvesting stage, parts of the plant, and extraction methods (Puvača et al., 2019). Source of inconsistency also relies on origin and type of EOs, the dosage level of EO supplemented in animal feed, the amount of FI, formulation, and digestibility of basal diet, and environmental conditions (Brenes and Roura, 2010; Dudareva, Pichersky, and Gershenzon, 2004). This review clarifies the current advancements in the utilization of EOs to possibly benefits in food animal production. Mode of action is summarized, including impacts on animal performance, control of pathogens, ruminal fermentation, and microflora.

ANTIMICROBIAL EFFECTS OF EOS

Plant and plant extracts have traditionally played a vital role in the wellbeing and healthcare of humans and animals as therapeutic agents for the treatment of many illnesses. Due to essence, flavor, antimicrobial, and preservative properties, plant secondary metabolites have been used by mankind since early history (Giannenas et al., 2020; Akram et al., 2019a; Jalal et al., 2019). EOs and their components are hydrophobic, a feature that allows them to penetrate the lipidic layer of bacteria resulting in the disturbance of cell osmotic pressure by interrupting membrane integrity and ion transport process (Florou-Paneri et al., 2019). EOs or their components sensitize the cell wall, causing significant membrane damages leading to the integrity collapse of membranes and biosynthetic machinery of the bacterial cell resulting in the leakage of vital cellular contents and death of bacterial cells. In detail, rapid dissipation of proton motive sources (hydrogen and potassium ion gradients) and depletion of the intracellular adenosine triphosphate (ATP) pool is seen through the declination of ATP synthesis and the increased hydrolysis. It results in the slowing down of bacterial growth by increasing permeability of the membrane and decreasing trans-membrane electric potential in the bacterial cell. When the bacterial cell tolerance level is passed, extensive loss of cell substances leads to cell death. Furthermore, the presence of hydroxyl group (OH) attached to a phenyl ring and its capability to discharge its proton are viewed as critical factors in disturbing normal ion transport across the cytoplasmic membrane and in deactivating microbial enzymes (Burt, 2004; Ultee et al., 2002). The previously described mode of action is more potent against gram-positive than gram-negative bacteria. The external cell wall of gram-negative bacteria is hydrophilic and hydrophobic components of EOs cannot easily infiltrate into the membrane. However, molecules of EOs with low molecular weight can interrupt the membrane integrity by passing the bacterial cell wall through diffusion with the assistance of membrane proteins or layer of lipopolysaccharides (Akram et al., 2019b; Giannenas et al., 2018).

EOs possess antimicrobial activity due to terpenoids and phenolic compounds (Florou-Paneri et al., 2019). Thyme and oregano inhibited the growth of pathogenic strains like *Salmonella enteritidis, Salmonella choleraesuis, Salmonella typhimurium,* and *Escherichia coli* (Peñalver et al., 2005), which is attributed to the phenolic components such as thymol and carvacrol. Moreover, Abdullah et al., (2015) studied the effects of clove bud oil and rosemary oil for their antimicrobial effects against multidrug-resistant strains such as *Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumannii,* and *Enterococcus feacalis.* EOs have also antimicrobial properties against zoonotic enteropathogenic strains like *Salmonella spp.* and *Escherichia coli O157:H7* (Guo et al., 2020; Olaimat et al., 2019), which shows that EOs can be used as alternatives to antibiotics in animal nutrition and production. Furthermore, EOs possess antimicrobial effects against gram-positive bacteria such as *Fusobacterium necrophorum, Trueperella pyogenes, Staphylococcus aureus,* and *Listeria monocytogenes* (Cho et al., 2020; Paiano et al., 2020) and gram-negative bacteria like *Escherichia coli* (Al-Mnaser and Woodward, 2020). In addition, EOs could be used against mastitis-causing bacteria (Amat et al., 2017; Zhu et al., 2016), respiratory pathogens (Akbari et al., 2018), and urinary tract infection (Ebani *et al.*, 2018). However, EOs showed effectiveness against viruses like *Melissa officinalis* EO was found effective against *Avian influenza virus* (Pourghanbari et al., 2016), while ajwain oil and *Artemisia arborescens* EOs showed antiviral activity against *Japanese encephalitis virus* (Roy, Chaurvedi, and Chowdhary, 2015) and *Herpes simplex virus type I and II* respectively (Sinico et al., 2005). Additionally, Govindarajan et al., (2016) observed that antilarval activity of the EO isolated from *Plectranthus barbatus* against larvae of the malaria vector *Anopheles subpictus*, the dengue vector *Aedes albopictus,* and the Japanese encephalitis vector *Culex tritaeniorhynchus.* Application of EOs in animal feed for health management, improvements in productivity and quality has proved a viable strategy, which is also the consumers' demand. The effects of EOs against bacteria, viruses, fungi, and protozoa are illustrated in Table 1. Dietary supplementation of EOs is an appropriate strategy of introducing natural antimicrobials in the body of animals that are entered, circulated in the body, and retained in tissues, which may provide help to prevent the lipid oxidation and microbial spoilage at their localized sites.

Table 1: Summary of studies testing antimicrobial activity of essential oils or their components against pathogenic microbes.

Essential oil or components	Species/group of microorganisms	References
Cinnamon	*Escherichia* coli and *Staphylococcus aureus*	Zhang et al., 2016
	Listeria monocytogenes	Abdollahzadeh et al., 2018
	Agrobacterium tumefaciens	Lee et al., 2020
	Escherichia coli	Kosariet al., 2020
	Bovine mastitis in organic dairy farming: *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hyicus, Staphylococcus xylosus* and *Escherichia coli*	Zhu et al., 2016
	Pseudomonas aeruginosa	Elcocks et al., 2020
Thyme	*Listeria monocytogenes*	Sarengaowaet al., 2019
	Escherichia coli (E. coli) O157:H7	Guoet al., 2020
	Streptococcus mutans	Abdel Hameed et al., 2020
	Staphylococcus aureus	Mohammed et al., 2020
	Streptococcus pyogenes	Maqbulet al., 2020
	Aspergillus flavus	Khaliliet al., 2015
Thyme & Cinnamon	*Salmonella* Species	Al-Nabulsiet al., 2020
	Salmonella species	Olaimatet al., 2019
Thyme & Oregano	*Listeria monocytogenes*	Cho et al., 2020

Pine oil	*Escherichia coli* O157:H7, *Listeria*, and *Campylobacter* species	Wells et al., 2015
Eucalyptus	Multi-drug resistant *Acinetobacter baumannii* *Escherichia coli*	Knezevicet al., 2016 Kareem et al., 2020
Eugenol	Verotoxin producing *Escherichia coli*	Ezzeldeenet al., 2015
Tea tree oil	Multi-drug resistant *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*	Oliva et al., 2018
MEO	Environmental mastitis: *Staphylococcus aureus, Staphylococcus chromogenes, Staphylococcus sciuri, Staphylococcus warneri, Staphylococcus xylosus* and *Escherichia coli* Bovine respiratory pathogen: *Mannheimia haemolytica* *Bovine endometritis: Escherichia coli, Fusobacterium necrophorum, Trueperella pyogenes, Staphylococcus aureus* Urinary tract infection: Multidrug-resistant strains of *Escherichia coli*, *Enterococcus* spp., *Candida albicans* and *Candida famata* *Leishmania, Plasmodium and Trypanosoma* species Arthropod disease vector: female *Ixodesricinus*	Fratini et al., 2014 Amat et al., 2017 Paiano et al., 2020 Ebaniet al., 2018 Le et al., 2018 Kulmaet al., 2017
Oregano	*Salmonella* species	Mohan and Purohit, 2020
Oregano & Rosemary	*Escherichia coli* O157:H7	Diniz-Silva et al., 2020
Oregano &Carvacrol	*Escherichia coli O23:H52*	Al-Mnaser and Woodward, 2020
Melissa oil	*Avian influenza A virus (H9N2)*	Pourghanbari et al., 2016
Ajwain oil	*Japanese encephalitis virus*	Roy et al., 2015
Plectranthus barbatus oil	Larvicides against malaria, dengue and Japanese encephalitis mosquito vectors	Govindarajanet al., 2016
Marrubium vulgare oil	Bovine reproduction system pathogen: *Trichomonas vaginalis*	Akbari et al., 2018
Arisaemafargesii	Larvicidal activity against *Aedes mosquitoes*	Huang et al., 2020
Myristica fragrans	*Escherichia coli*, *Staphylococcus aureus*	Kiarsiet al., 2020

MEO = mixture of essential oils

EFFECTS OF EOS ON THE DIGESTIVE SYSTEM OF MONOGASTRIC ANIMALS

Dietary supplementation of EOs has positive effects on animal health, gut microflora, intestinal morphometrics, enzymatic activity, and growth performance parameters that have been studied comprehensively in Table 2. In general, EOs seem to stimulate beneficial bacteria, inhibit pathogenic microbes, regulate enzyme activities and execute beneficial effects on gut villi with inducing positive effects on BWG, FCR, and FI (Abbasi et al., 2019; Barbarestani et al., 2020; Park and Kim, 2018; Yang et al., 2019). Beneficial microbes like *Lactobacilli species (sp.)* trigger the local intestinal immune system by releasing the peptides of low molecular weight, which increase the resistance against diseases (Muir et al., 2000). Furthermore, a high number of *Lactobacilli sp.* decrease the pathogenic microbes through developing the colonization resistance by modifying the receptors used by pathogens (Rinttilä and Apajalahti, 2013; Adil and Magray, 2012). EOs further showed improvements in averaged daily gain, growth performances, carcass quality and reduced cholesterol level in broilers, quails, and pigs (Attia, Bakhashwain, and Bertu, 2017; Fathi et al., 2020; Mercati et al., 2020; Placha et al., 2019; Wade et al., 2018). Moreover, they helped poultry in fighting against diseases such as Newcastle disease, Infectious bursal disease, and avian influenza and coccidiosis (Ahmadian et al., 2020; Lee et al., 2020). EOs also increased the immunity and antioxidant capacity in heat stress periods (Eler *et al.*, 2019; Sariözkan et al., 2020). In layer hen, along with improving growth performance characteristics, EOs improve egg quality and shell related parameters (Abo Ghanima et al., 2020; Beyzi et al., 2020; Yalçin et al., 2020). As documented in the literature, EOs exhibit antimicrobial activity against *Escherichia coli* (Park and Kim, 2018)*, Clostridium perfringens* (Cho et al., 2014), *Salmonella typhimurium* (Ahmed et al., 2013), and prevent the adhesion, colonization, and proliferation in the gut of broiler. The increased number of beneficial bacteria and decreased number of pathogenic bacteria maintain the proper bacterial balance in the intestine seem to improve the intestinal absorptive capacity by improving the ability of epithelial cells to regenerate villi (Pathak et al., 2017).

Table 2: Effects of essential oils/components on digestive system and growth performance parameters in mono-gastric animals.

Essential oil and components	Dosage level	Observations	References
Broilers			
Thyme	1.0-2.0 g/kg	Improvement in FCR and Immunity during HS	Attia et al., 2017
	0.5-1 g/kg	Increase in BWG, FI and improvement in FCR	Pournazariet al., 2017
	300 mg/kg	Increase of digestive enzyme activities, intestinal morphometrics and immunity	Yang et al., 2018
	100 mg/kg	Increased BWG, improved FCR, livability and profit	Wade et al., 2018
	5 g/kg	Improvement of BWG, FI, FCR. Beneficial effects on cholesterol , immunity and antioxidant status	Ismail et al., 2019
	0.1 %	Increased meat quality and antioxidant status in breast muscle	Plachaet al., 2019
	150-200 mg/kg	Improved growth performance and immune responses in HS	RafatKhafaret al., 2019
	300 mg/kg	Reduced adverse effects of HS	Sariözkanet al., 2020
Sumac and Thyme	1, 2 & 3 %	Reduce fat content and improve disease responsiveness, antiviral effects against ND and AI.	Ahmadian et al., 2020
Thyme and Carvacrol	60 and 120 mg/kg	Showed anticoccidial effects	Lee et al., 2020
Thyme oil and Black cumin oil	250 and 100 mg/kg	Positive effects on intestinal health	Aydin and Yildiz, 2020
Thyme and Peppermint	100 mg/kg each	Improved ADG, FCR, carcass yield and decreased cholesterol level	Hassan, 2019
	NS	Increased BW, WG and immune response	Witkowskaet al., 2019
Oregano	150 mg/kg	Increased ADG, ADFI and antioxidant status	Riet al., 2017
	200-600 mg/kg	Improved performance and meat quality as increased breast meat redness and reduced yellowness	Cázares-Gallegos et al., 2019
	NS	Beneficial effect on the growth performance	Hn et al., 2019
	300-900 mg/kg	Improved performance, carcass yield and immunity	Eleret al., 2019
Oregano and Thyme	4% each	Improvement of WG, immune parameters and intestinal morphology	Parvizi et al., 2020
MEO	0.03%	Improvement of total tract retention of DM, increase of LAB and reduction of E.coli	Park and Kim, 2018
	0.01%	Improvement of BW, FCR and LAB	Ruben et al., 2018
	NS	Improved immunity and showed antiviral effects against ND and IBD	El-Shall et al., 2020
Cinnamon	500 mg/kg	Increased villi height and immunity, reduction of *Salmonella* and *Clostridium* counts	Pathak et al., 2017
	NS	improved the immune status, antioxidant ability and cecal microbiota	Yang et al., 2019
Rosemary	300 mg/kg	Improvement in FCR, immunity and concentration of Se in liver and breast muscles of broiler	Mohammadi et al., 2019
	0.5-1.0 g/kg	Beneficial effect on lipid profile, immunity, antioxidant status	El-Gogary, 2020
Rosemary, Thymus & Satureja	300 ppm	Improved immune responses, antioxidants and intestinal microflora	Abbasiet al., 2019
Rosemary & Thyme	5-10 g/kg each	Significant effect on live BW, FI and dressing percentage	Tayeb et al., 2019
Lavender oil	600 mg/kg	Increased growth performance, intestinal morphometrics, villi height, antioxidant status and gut bacteria balance, reduced E. coli	Barbarestaniet al., 2020
Laying hen			
Oregano	50-250 mg/kg	No effect on FI, FCR, egg production and egg shell characteristics	Cufadar, 2018

	50-200 mg/kg	Enhanced immune response	Migliorini et al., 2019
Thyme	300 mg/kg	Improvement in antioxidant status during HS period	Beyziet al., 2020
	2%	Showed hypolipidemic and antioxidative effects along with improved immunity without effecting performance and egg quality	Yalçinet al., 2020
Rosemary & Cinnamon	300 mg/kg each	Significant better egg production and weight, Haugh unit, FI, FCR, blood cholesterol, immunity, and antioxidant parameters	Abo Ghanimaet al., 2020
Quil			
Thyme	200-400 ppm	Improved FCR	Dehghaniet al., 2018
	150-450 mg/kg	Increased BW, ADG, FI and antioxidant status	Gumuset al., 2017
Eucalyptus	0.1%	Enhanced productive performance, eggshell quality, immunocompetence and reduces number of broken eggs	Fathiet al., 2020
Cinnamon and Ginger oil	0.5-0.1 ml/kg	Improvements in ADG and FCR	Ahmed et al., 2019
MEO	0.33-1.0 ml/L	Improved BW, FCR, villi height and intestinal health	Masood et al., 2020
	600-900 g/ton	Improvements in growth hormone, growth performance and intestinal histomorphology	Maty and Hassan, 2020
Pigs			
Thymol &Carvacrol	30 mg/kg	Improvement of ADG, apparent digestibility of DM, crude protein, gross energy and enzymatic activity in intestine. Increased LAB	Xu et al., 2018
MEO	100 mg/kg	Enhanced growth performance and decrease diarrhea prevalence through increases in antioxidative capacity.	Tian and Piao, 2019
Plant EO	NS	Improvement of BW, growth performance, immunity and antioxidant status	Su et al., 2018
	50-200 ppm	Improvements in regulation of growth and intestinal health	Su et al., 2020
Oregano	NS	Increased antioxidant action and can be used as antimicrobial agent to prevent antimicrobial resistance	Mercatiet al., 2020
	2000 ppm	Increased carcass performance and consumer acceptability.	Janacua-Vidaleset al., 2019
	400 g/ton	Increased Bifidobacterium and Bacillus species to improve immune status	Pu et al., 2020

MEO = Mixture of essential oils, FCR = feed conversion ratio, HS = heat stress, BWG = body weight gain, FI = feed intake, ND = Newcastle disease, AI = avian influenza, ADG = average daily gain, BW = body weight, WG = weight gain, ADFI= average daily feed intake, DM = dry matter, LAB = lactic acid producing bacteria, IBD = infectious bursal diseas, NS = not specified

EFFECTS OF EOS ON RUMEN FERMENTATION

Ruminant animals are producing high-quality protein from low-quality feed resources due to their symbiotic relationship with ruminal microflora. The efficiency of rumen metabolism is also associated with environment-polluting waste products. Inefficiency in rumen fermentation leads to energy and protein losses in the form of methane and ammonia gas production. Methane is the main constituent of greenhouse gas which plays 21 times more potential role in global warming than carbon dioxide (Bodas et al., 2012). Moreover, 2-12% of gross energy intake dissipates into enteric methane mitigation in ruminants depending upon feed intake and type of diet (Benchaar and Greathead, 2011). It can be therefore determined that a decrease in methane emission with the dietary supplementation of EOs is favorable both for the animals and the environment. EOs also possess a significant influence on protein metabolism and reduce ammonia production by inhibiting the deamination of amino acids (AA), possibly through the suppression of HAP at the level of adhesion and colonization (Benchaar and Greathead, 2011; McIntosh et al., 2003).

Several EOs (oregano, cinnamon, eucalyptus, rosemary, clove oil, garlic oil, and peppermint oil) have already been tried *in vitro* and *in vivo* in animals to reduce methane and ammonia production (Baraz et al., 2018; Cobellis et al., 2015, 2016; Hamdani et al., 2019; Tomkins et al., 2015). EOs do not affect rumen fermentation at low doses, whereas, these compounds inhibit the target microbial species as well as rumen microbes at high doses. EOs might selectively discourage the methanogens and HAP bacteria at low doses, but the high concentration of EOs overwhelm all the microorganisms (Cobellis et al., 2016; Wallace, 2004). Mitigation of methane and ammonia occurs at high doses and it is frequently associated with a decrease in dry matter (DM) degradability, feed digestion, total VFA production and rumen fermentation (Vendramini et al., 2016; Cobellis et al., 2016; Hristov et al., 2013). EOs (oregano, cinnamon, eucalyptus, and rosemary) both individually and in combination reduced methane and ammonia production (Cobellis et al., 2016). Zhou et al., (2020) also revealed that supplementation of oregano EO at 13-130 mg/liter potentially reduced the methane production. Various investigations demonstrated that the composition and inclusion level of EOs could affect the ruminal N metabolism. Cinnamon bark inhibited the ammonia production by 43.9% and 59.3% reduced by the combination of cinnamon, oregano, and rosemary leaves (Cobellis et al., 2015). Patra and Yu, (2012) reported that EOs of oregano and clove decreased the ammonia production more potentially *in vitro* when compared with garlic, eucalyptus and, peppermint EOs. Multifaceted relations happen among EOs, feed, and host, thus correlation of the results from feed degradability, rumen fermentation features, and microbiome dynamics could provide more information for the development of effective mitigation technologies.

Total VFAs production is little affected (Patra and Saxena, 2010) or decrease due to high concentrations of EOs in the diet (Baraz et al., 2018). Dietary supplementation of clove and thyme EOs at 2ml per day in sheep increased the total VFA concentrations (Abeer et al., 2019). Some EOs and their major constituents shift molar proportions of VFA i.e. decrease in acetic acid and an increase in the propionic acid proportion which is nutritionally favorable (Ribeiro et al., 2019; Silva et al., 2020).

Variations in results among *in vitro*, *in situ*, or *in vivo* studies can be attributed to numerous variables such as diet (forage: concentration ratio), pH (more potent action at low pH) time (adaptation period), and EOs composition. The lack of effects of EOs on rumen metabolism in long-term studies as compared to short-term studies could be due to adaptation of ruminal microbes to EOs and the obvious difficulty in predicting the dose rate of dietary supplementation of EOs. Long term exposure of EOs may lead to adjustments in rumen micro-organisms, and it is convincible that some EO compounds are subjected to degradation by rumen microbial populations (Abdallah Sallam et al., 2011). Cardozo et al., (2004) examined the effects of cinnamon, garlic, and anise oils at different doses such as 7.5 mg/kg and 0.22 mg/liter of DM on continuous culture. They noticed the progression in the VFA profile during the initial six days, however no effects from that point because of microbial adaptation to EOs. EOs containing phenolic compounds as an active compound exert more pronounced antimicrobial effects than others (Patra and Yu, 2012). Although EOs in high doses could exert positive effects in vitro on rumen fermentation, these doses result in negative implications on feed palatability, digestion, and animal productivity, when applied *in vivo* (Yang et al., 2010; Beauchemin and McGinn, 2006). At the same time, the levels of EOs that have elicited favorable fermentation responses in vitro are far too high for *in vivo* applications due to their possible toxic effects and high cost.

Besides, very few data available on the effects of EOs on DM intake, milk production, composition, and body growth of ruminants. Oregano increased the rumen fermentation, FI, DM digestibility, and feed efficiency along with reduction in methane production and ammonia nitrogen (Tapki et al., 2020; Zhou et al., 2020). Moreover, the addition of a mixture of essential oils (MEO) in the diet increased the average daily gain, live weight, FCR, and nutrient digestibilities (Giller et al., 2020). They also increased gut health, immunity, and prevented the animals from diarrhea and other diseases (Campolina et al., 2020; Liu et al., 2020). In addition, EOs increased milk production, milk fat, and carcass characteristics (Hart et al., 2019; Silva et al., 2020; Wang et al., 2020). Supplementation of EOs could increase conjugated linoleic acid, a health-promoting fatty acid in milk fat by suppressing the bacteria involved in biohydrogenation (Bayat et al., 2015). Rivaroli et al., (2016) recommends MEO (oregano, garlic, lemon, rosemary, thyme, eucalyptus, and sweet orange) at 3.5 g/day in feedlot animals to decrease the lipid oxidation. Table 3 shows effects on growth performance parameters along with effects on methane production, total VFA concentrations, and rates (i.e., acetate to propionate ratio), animal health, performance, and quality characteristics of animal products.

Table 3: Effects of essential oils or their components on rumen characteristics and performance of ruminants.

Essential oil or components	Dosage level	Observations	References
Cattle			
Oregano	100-150 mg/L	Improved FE, growth performance, health status. Reduced diarrhea incidents and lower farm costs	Tapkiet al., 2020
	13-130 mg/L	Increased DM, NDF and ADF digestibility. Decreased AN, MP and alter VFA concentration	Zhou et al., 2020
	4 g/day	Alter ruminal microbiota	Zhou et al., 2019
	50 mg/kg	No effect on RF, ND, MP, MY and MF	Benchaar, 2020
	10 g/day	Improved FE	Bosco Stivaninet al., 2019
BEO	1 g/day	Immunity improvement and a decrease morbidity of neonatal diarrhea in pre-weaning phase	Campolinaet al., 2020
	3.5 g/day	No effect on carcass quality. EOs can be added in low amount without affecting meat quality	Rivaroliet al., 2020
	150 mg/kg	Increased NDF digestibility and N utilization	Teobaldoet al., 2020
	150 mg/kg	Increased NDF and OM digestibility, MY and MF. Reduced A:P ratio	Silva et al., 2020
	4 g/day	Improved ADG, DM intake, FE	Souza et al., 2019
	1000 mg/day	No effect on rumen microbiota	Schärenet al., 2017
	1 g/day	Increased MY and reduced MP	Hart et al., 2019
	1 g/day	Increased MY and FE	Elcoso et al., 2019
MEO	25 g/day	Increased FI. No effects on milk composition and antioxidant capacity	Gilleret al., 2020
	1 g/day	Improved carcass quality	Wang et al., 2020
	NS	Improvements of ADG, FCR, ND, calf growth, ruminal development, gut health, and immunity	Liu et al., 2020
	50-100 ul/L	Improved DM digestibility and microbial protein yield. Reduced MP	Davoodiet al., 2019
	1 g/day	Improved meat quality attributes	Pukrop et al., 2019
	25 mg/kg	Improved MY, udder health and immunity	Salem et al., 2019
	100 µl/L	Reduced MP, increased microbial protein synthesis and RF	Kurniawatiet al., 2020
	3.5 g/animal/day	Decrease in the lipid oxidation.	Rivaroli et al., 2016
Thyme	100 ul/L	Decreased AN, VFA concentration and MP	Baraz et al., 2018
	100 g/day	Increased MY and reduced MP	Hamdaniet al., 2019
Clove & Rosemary	2 g and 4 g / animal/day	No effect on carcass quality. Affect oxidation	de Oliveira Monteschioet al., 2017
Coriander oil	14 mL/cow/day	Increase of FI, ND and MY. Decreasein ruminal AN concentration	Matloupet al., 2017
Cashew and caster	2 g/day	No effect on FI and N digestibility. Alter ruminal pH	Coneglianet al., 2019
Buffaloes			
Ajwain oil	1-2 ml/day	Increased DM intake, ADG and protein metabolism	Pawaret al., 2019
	0.05%	Reduced MP, A:P ratio and improved ND	Wadhwa and Bakshi, 2019
Eucalyptus	20-120 ul/40ml	Reduced MP	Singh et al., 2019
	NS	No effect on FI, ND, Ruminal pH, temperature and BUN. Increase of total VFA concentration. Decrease of ruminal AN, protozoal, proteolytic bacteria, MP and A:P ratio	Thao et al., 2015
	2 mL/day	No effect on FI, ND, N utilization, total VFA concentration. Decrease of MP and A:P ratio. Reduction of protozoal population	Thao et al., 2014
Sheep			
Thyme	1.25g/kg	Increased RF and N metabolism. Decreased A:P ratio	Ribeiro et al., 2019

Clove	2 ml/day	Improved ND and carcass characteristics	El-Essawyet al., 2019
Clove and Thyme	2 ml/day each	Increased MY, MF, VFA and antioxidant capacity. Reduced cholesterol	Abeer et al., 2019
Orange peel	300-450 mg/kg	Increase of FI, antioxidant status and MF	Kotsampasiet al., 2018
Rosemary	0.3-0.6 ml/day	No effect on DMI, and growth. Increase of PUFA and sensorial attributes in meat	Smetiet al., 2018
Garlic oil	62.5 mg/L	No effect on ADG, performance, FCR, ND, calcium and phosphorus blood concentration. Improvement of TDN and digestible CP conversion ratio	El-Katcha, Soltan, and Essi, 2016
MEO	1.6 mL/day	No effect on ruminal pH, VFA concentration, MP, A:P ratio and blood profile. Decrease of ruminal AN	Khateri et al., 2017
Chavil EO	250-750 mg/kg	Decreased saturated fatty acids and increased antioxidant capacity of meat	Parvaret al., 2018
Functional EO	2-6 g/day	Decreases FI without negatively affecting nutrient fermentation and usage	Michailoffet al., 2020
Red pepper EO	0.14-0.42%	Improved carcass characteristics	Bertoloniet al., 2020
Goat			
Callistemon viminalis oil	100-200 mg/kg	Improvement of DM intake, ND, N utilization and biochemical parameters	Mekuikoet al., 2018
Rosemary	600 mg/kg	No effect on DM, OM, CP and NDF digestibility. Increase of MY, MF and protein contents	Smetiet al., 2015
	100-400 mg/kg	No effect on ADG, hematological parameters. Increased immunity	Shokrollahiet al., 2015
Oregano & linseed	3% and 0.6 %	Improvements of carcass quality and antioxidation. No effects on performance parameters	Rotondiet al., 2018
Juniper	0.4-2 ml/kg	No effect on FI, LWG, ruminal pH, VFA concentration, fecal pH. Increase of FE and antioxidant status	Yesilbaget al., 2017
MEO	2 mg/kg	Increased ADG and improved phenotypes (cashmere fiber traits, carcass weight, and meat quality)	Lei et al., 2019
Fennel EO	100-1000 ug	Decreased MP, AN	Cheshmehgachiet al., 2019

MEO = mixture of essential oils, BEO = blend of essential oils, FE= feed efficiency, DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, AN = ammonia nitrogen, MP = methane production, VFA = volatile fatty acids, RF = rumen fermentation, ND = nutrient digestibility, MY = milk yield, MF = milk fat, N = nitrogen, A:P = acetate to propionate, ADG = average daily gain, FI = feed intake, FCR = feed conversion ratio, BUN = blood urea nitrogen, PUFA = poly unsaturated fatty acids, TDN = total digestible nutrients, CP = crude protein, OM = organic matter, NS = not specified

ANTIOXIDANT EFFECTS OF EOS

The most important purpose of EOs is to minimize the pathogenic microbes and decrease the phenomenon of lipid oxidation. Oxidation of lipids and free radical production are natural processes that influence the membrane integrity, interrupt the cell transport channels and function of cell organelles. Lipid content of membrane particularly phospholipids is more inclined to oxidative damage, which is related to the level of unsaturation of fatty acids (UFAs). Polyunsaturated fatty acids (PUFA) are responsible for keeping up cell membrane respectability including fluidity and permeability. Hydroperoxides (ROOH) formation occurs because of reaction between peroxy radicals and polyunsaturated FAs resulting in the formation of non-radical aromatic compounds that adversely affect the carbohydrates, protein, lipids, and vitamin contents and limit the nutritional value and shelf life of animal products. The EOs as an antioxidant have various modes of action to reduce lipid oxidation. One of the possible mechanisms of action is that they block the chain initiation, start the hydrogen abstraction, act as free radical scavenger and terminators, bind the transition metal ions, and stop the formation of singlet oxygen (Tongnuanchan and Benjakul, 2014). Several EOs possess phenolic compounds up to 85% of their composition. In phenolic compounds, carvacrol, eugenol, and thymol are the active components that act as primary oxidants and effective free radical scavengers (Bakkali et al., 2008). Antioxidants work in three stages: initiation, propagation, and termination. The presence of hydroxyl group (-OH) in antioxidant compounds usually acts as a hydrogen donor, inactivates the free radicals generated from the lipid oxidation. They scavenge the free radicals by donating electrons to them, this feature makes them potentiated anti-oxidant that prevents other compounds from oxidizing (Coma, 2012). It results in the development of new radicals, which are unable to extract the hydrogen (H) atoms from unsaturated FAs (Coma, 2012). Hence, these subsequent radicals can react with similar radicals or free radicals leading to the formation of non-radical species (Jayasena and Jo, 2014). In this way, phenolic compounds can counteract lipid oxidation, act as oxidative chain inhibitors, and protect the animal products from oxidative damage.

Animal diet can play an important role to inhibit the free radical production in organisms and their derived products. The addition of EOs in the diet of animals is a simple and efficient approach to incorporate natural anti-oxidant compounds into lipidic layers of membrane (Table 4). In this way, they can inhibit lipid oxidation more effectively and prevent oxidative losses of animal products compared to postmortem addition (Decker and Park, 2010; Govaris et al., 2004).

Table 4: Summary of studies testing antioxidant activity of essential oils or their components in food processing

Essential oil	Dosage level/ concentration applied	Product	Effect	SP	SL	References
Oregano	0.2%	Rabbit meat	+		+	Cardinaliet al., 2015
	0.125-3.0 ml/kg	Rainbow trout	NE			Dileret al., 2017
	2000 ppm	Pig meat	+	+		Janacua-Vidaleset al., 2019
	NS	Pig meat	+	+		Mercatiet al., 2020
	200-600 mg/kg	Broiler meat	+	+		Cázares-Gallegos et al., 2019
Oregano & linseed	3% and 0.6 %	Goat meat	+	+		Rotondiet al., 2018
Rosemary	200-400 mg/kg	Lamb meat	+		+	Ortuñoet al., 2014
	300 mg/kg	Broiler meat	+	+		Mohammadi et al., 2019
Rosemary and Thyme	5-10 g/kg each	Broiler meat	NE			Tayeb et al., 2019
Rosemary and Cinnamon	300 mg/kg each	Layer meat and egg	+	+		Abo Ghanimaet al., 2020
Thyme and Peppermint	100 mg/kg	Broiler meat	+	+		Hassan, 2019
Thyme	0.125%	Fresh chicken sausage	+		+	Sharma et al., 2017
	0.1%	Broiler meat	+	+		Plachaet al., 2019
	300 mg/kg	Layer meat and egg	+			Beyziet al., 2020
	2%	Layer meat and egg	+	+		Yalçinet al., 2020
	150-450 mg/kg	Quil meat	+	+		Gumuset al., 2017
	600 mg/kg	Broiler meat	+	+		Onel and Aksu, 2019
Thyme & Clove	4 MIC and 2 MIC respectively	Minced beef	+		+	(Zengin and Baysal, 2015
Clove	0.25%	Fresh chicken sausage	+		+	Sharma et al., 2017
	2ml/d	Sheep meat	+	+		El-Essawyet al., 2019
Basil EO	0.062, 0.125 and 0.25%	Beef burger	+	+	+	Sharafati Chaleshtori et al., 2015
	2 and 4%	Cattle meat	+	+	+	Falowoet al., 2019
	0,25, 0.50, 0.75%	Mutton nuggets	+	+	+	Kumar et al., 2018
Sage oil	0.05, 0.075, 0.1 µL/g	Pork fresh sausages	+	+		Šojićet al., 2018
Chavil EO	250-750 mg/kg	Sheep meat	+	+		Parvaret al., 2018
MEO	750-2000 mg/kg	Broiler meat	+	+		(Mountzouris et al., 2020)
	250-750 ml/1000 L	Broiler meat	+	+		Tekceet al., 2020
	25 g/day	Cattle meat	+	+		Giller et al., 2020
	1 g/d	Cattle meat	+	+		Pukrop et al., 2019
	2 mg/kg	Goat meat	+	+		Lei et al., 2019

MEO = blend of oils, SP = sensory properties, SL = shelf life, NE = no effect

CONCLUSION

The growing pressure on the livestock industry is to limit the application of chemical feed additives particularly antimicrobial agents as growth promoters have started a new investigation to discover the safe and effective substitutes. A variety of different biologically active agents including EOs proved themselves as multifunctional feed supplements for animals. The EOs and their constituents possess the remarkable potential to influence the gut-microbiota, rumen fermentation and avoid lipid oxidation results in the improvements in growth performance parameters and quality characteristics of animal products. Whereas, their potential and efficacy in livestock production have not yet been determined to be steady and indisputable and some concerns should be investigated before their business application. For instance, an ideal concentration of EOs according to their chemical composition and type, ought to be established, since their application at high doses can impose undesirable effects on living organisms. Dietary supplementation of EOs is safe to use but their mode of action, pharmacokinetics, and pharmacodynamics are still unclear. Simultaneously, the good effects of dietary supplementation of EOs ought to be legitimized the extra expense of their application. A further demonstration of the above inquiries is needed for the regular application of EOs in animal production. In this way, it may be possible to formulate animal feed that optimizes animal efficiency. EOs besides being a promising approach as drug candidates in modern medicine, their dietary supplementation in food (soft drinks and food confectionary) and feed industry (growth promoters, antimicrobials, and antioxidants) can also be action-oriented approach in modern nutrition.

CONFLICT OF INTEREST

None declared by the authors.

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