Review

Enrichment of Animal Diets with Essential Oils—A Great Perspective on Improving Animal Performance and Quality Characteristics of the Derived Products

Panagiotis E. Simitzis

Department of Animal Breeding and Husbandry, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, 75 Iera Odos, Athens 11855, Greece; pansimitzis@aua.gr; Tel.: +30-21-0529-4427; Fax: +30-21-0529-4442

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Abstract: Food industry operates in a competitive market and is continually facing challenges to retain or even increase its market share. Consistent high-quality animal products are required to maintain consumer confidence and consumption. Enrichment of foods with bioactive compounds such as the essential oils appears to improve quality characteristics of the derived products and protects consumers against oxidation and bacterial spoilage effects. Synthetic additives are nowadays questioned due to their suspected carcinogenic potential, and therefore extensive research has been undertaken to identify safe and efficient alternatives. Aromatic plants and their respective essential oils belong to natural products and are generally used in pig, poultry, rabbit and ruminant nutrition. The inclusion of essential oils in livestock diets is nowadays becoming a common practice, since dietary supplementation has been proven a simple and convenient strategy to effectively inhibit the oxidative reactions or microbial spoilage at their localized sites. A wide range of essential oils contain bioactive compounds that have the potential to act as multifunctional feed supplements for animals including effects on growth performance, digestive system, pathogenic bacterial growth and lipid oxidation. However, further studies are needed to clarify their exact action and establish their regular use in animal production.

Keywords: essential oils; growth performance; animal products; microbial spoilage; oxidation

1. Introduction

The growing public concern over the potential health risks and environmental impacts caused by the excessive use of antibiotics as growth promoters in animal production has led to a ban of their dietary application within the European Union since 2006 with the intention to minimize the transmission and the proliferation of resistant bacteria via the food chain. However, industries involved in the animal production chain, including the primary producers, processors, distributors, and retailers are constantly searching for efficacious, safe and cost effective substances with similar properties. As a result, natural feed supplements derived from plants such as the essential oils (EOs) have been examined as alternatives in animal production for improving growing performance parameters and the quality characteristics of the derived products (meat, milk and eggs) [1].

EOs are quite complex mixtures constituted by several components, hence resistance is less likely to become a problem compared to a single synthetic compound. They mainly consist of terpenoids and a variety of low molecular weight aliphatic hydrocarbons (phenols, aromatic aldehydes, etc.) [2]. EOs have the potential of a possible therapeutic exploitation in a variety of conditions in animal production: they enhance production of digestive secretions, stimulate blood circulation, exert antioxidant properties, reduce levels of pathogenic bacteria counts, mitigate the levels of fermentation...
products (ammonia and biogenic amines), enhance precaecal nutrient digestion, improve the intestinal availability of essential nutrients for absorption and relieve animal from immune defense stress (less activity of the gut-associated lymphatic system) [3–5]. However, the mechanisms underlying these functions have not yet been thoroughly elucidated.

Factors such as geographical location, harvesting period, plant part used (seeds, leaf, root or bark) and method of isolation (cold expression, steam distillation, extraction with non-aqueous solvents, etc.) modify the chemical composition of the essential oil derived from the respective plant and may introduce substantial compositional differences between preparations from the same plant species. Source of variability is also the type and origin of the EO, the level of EO that is included in the animal diet, the composition and the digestibility of the basal diet, the level of feed intake and hygiene and environmental conditions [4,6].

2. Classification, Structure and Metabolism of EOs

Essential oils are volatile secondary metabolite fractions mainly extracted by steam distillation (but also cold pressing, fermentation and enfleurage) that have been extensively used in the cosmetic but also in the food industry. The most important active compounds of EOs are categorized into two chemical groups: (mono- and sesqui-)terpenoids and phenylpropanoids that originate from different precursors of the primary metabolism and are further synthesized through separate metabolic pathways. Terpenoids (limonene, thymol, carvacrol, linalool, etc.) are characterized as deriving from an isoprene unit, namely a basic structure of five carbons (C$_5$H$_8$) through the mevalonate pathway [7]. On the other hand, phenylpropanoids (cinnamaldehyde, eugenol, anethole, etc.) derive mainly from the phenylalanine that is synthesized by the shikimate metabolic pathway [8].

3. Antimicrobial Effects of EOs

Control of animal products spoilage and pathogenic bacteria has been mainly achieved by the use of synthetic chemical agents in previous decades. The applications of these compounds are nowadays limited due to questions regarding their potent undesirable aspects such as carcinogenicity, acute toxicity, teratogenicity and slow degradation periods. Public awareness has therefore generated interest in the use of more naturally occurring compounds with a broad spectrum of antimicrobial properties that improve the quality characteristics and shelf life of animal products [9]. Essential oils belong to the category of natural antimicrobials and have already been extensively studied for their antimicrobial activities in food systems [2,10,11]. Specific circumstances in food systems such as lipid content, proteins, water activity, pH and enzymes can potentially diminish the efficacy of EOs [10].

EOs and their components are hydrophobic, a characteristic that enables them to partition lipids in the bacterial cell wall and mitochondria, leading to their accumulation in the lipidic layer and a disruption of the membrane integrity and ion transport processes, and resulting in disturbances of the cell osmotic pressure. In detail, a rapid dissipation of H$^+$ and K$^+$ ion gradients (proton motive sources) and depletion of the intracellular ATP pool is observed through the reduction of ATP synthesis and the increased hydrolysis. As a result, the trans-membrane electric potential in bacterial cell is reduced and the proton permeability of the membrane is increased slowing down bacterial growth. When the bacterial tolerance threshold is passed, the extensive loss of cell contents or critical molecules and ions leads to cell death [10]. Moreover, the existence of the hydroxyl group attached to a phenyl ring and its ability to release its proton are considered as crucial factors in disrupting normal ion transport across the cytoplasmic membrane and in inactivating microbial enzymes [10,12].

The above mechanisms of action are more effective against gram-positive bacteria because the hydrophobic compounds of EOs can directly interact with the cell membrane. On the other hand, the external cell wall of gram-negative bacteria is hydrophillic and as a result the hydrophobic (or lipophillic) compounds of EOs cannot easily penetrate into the bacterial membrane [10,13]. However, low molecular weight molecules of EOs can cross the bacterial cell wall by diffusion
through membrane proteins or through the layer of lipopolysaccharides resulting in the disruption of membrane integrity [2].

Post mortem application of EOs in food systems contributes to the extension of shelf life by limiting the spoilage of foodborne pathogens [11]. However, during the last decade, dietary supplementation with EOs is constantly gaining ground since it is a simple and convenient way of introducing natural antimicrobials that enter the circulatory system, and are further distributed and retained in tissues. For example, dietary supplementation with the EOs derived from Oregano sp. could be used for improving the microbial hygiene of turkey breast fillets [14] or that of whole carcasses by reducing the microbial load of total viable bacteria and of specific pathogens as shown in broilers [15] and rabbits [16].

3.1. Effects of EOs on the Digestive System and the Gut Microbiota of Swine and Poultry

The effects of EOs dietary supplementation on gut microflora, morphology, enzyme activity and growth performance parameters have been already extensively examined (Table 1). In general, EOs appear to suppress harmful microorganisms, stimulate beneficial microbes such as *Lactobacillus* spp., regulate the activity of enzymes and protect gut villi, however without inducing significant positive effects on body weight gain. On the other hand, feed conversion ratio is generally improved. Lactobacilli are a group of bacteria that have long been known for their ability to activate the intestinal immune system and increase the resistance to diseases, in part through the release of low-molecular weight peptides which induce immune activation [17]. At the same time, the increased Lactobacilli counts contribute to the colonization resistance against pathogenic microbes by modifying the receptors used by them [18,19]. As shown by the literature, EOs display antimicrobial action against *Escherichia coli* [20], *Clostridium perfringens* [21] and *Eimeria tenella* [22] and prevent their adhesion, colonization and proliferation in the gut of broilers. The decreased numbers of pathogenic bacteria in the gut and the maintenance of a proper bacterial balance between the number of beneficial and harmful bacteria in the intestine appear to improve the ability of epithelial cells to regenerate villus and thus enhance intestinal absorptive capacity [23].

The gut is a pivotal organ system that mediates nutrient uptake and use by the animals. As presented in Table 1, essential oils beneficially affect the ecosystem of gastrointestinal microflora by controlling potential pathogens, alleviating the oxidative stress caused by them and stabilizing gut microbiota. Improved intestinal health further enhances availability of essential nutrients for absorption (increased villus length and gut surface) and animal growth performance parameters could therefore be improved [3,5,24]. Moreover, digestive secretions (saliva, bile, mucus, etc.) and enzyme (trypsin, amylase, lipase, etc.) activity are enhanced partially through the irritation of the epithelial tissues and the reduction in the depth of the crypts in the ileum, resulting in an increased gastric retention time of the ingested feed and a better nutrient absorption [25,26].

The exact reasons for the discrepancies shown in Table 1 are speculative but they could be attributed to different type and levels of essential oil, the variability in the concentration of the major bioactive compounds among the different parts (barks, leaves, flowers, etc.) of the plant used, the period of supplementation and the animal species. Moreover, the variability in the efficacy of essential oil on performance of farm animals could also be a result, among others, of the basal diet composition, feed intake level, hygienic standards and environmental conditions [4].
Table 1. Effects of essential oils or their components on gut microflora, morphology, enzyme activity and growth performance parameters in monogastric animals.

| Essential Oil or Component | Level        | Animal | Effects                                                                 | Reference |
|----------------------------|--------------|--------|-------------------------------------------------------------------------|-----------|
| Artemisinin                | 17 ppm       | Broilers | Reduction of oocyst output and lesion scores attributable to *Eimeria tenella.* | [27]      |
| BEO (thymol, eugenol and piperine) | 100-200 mg/kg | Broilers | No effect on intestinal numbers of *C. perfringens*, GP and FCR. Reduction of FBW. | [28]      |
| BEO (thymol, eugenol and piperine) | 100 mg/kg   | Broilers | No effect on FL, BWG, FCR, CT and ileal bacterial count (C. perfringens and Gram- bacilli). Increase of ileum length and ileal villi height. | [29,30]  |
| BEO (carvacrol, thymol, eucalyptol, lemon) | 125–500 mg/kg | Broilers | Improvement of BWG and FCR (125 or 250 mg/kg). Reduction of *Salmonella Heidelberg* colonization in crops (500 mg/kg). No effect on *Salmonella Heidelberg* caecal or faecal counts. | [31]      |
| BEO (cinnamonaldehyde and thymol) | 100 mg/kg   | Broilers | No effect on ADG, FI, gut morphology and ileal bacterial count. Improvement of FCR and apparent ileal nitrogen digestibility. | [32]      |
| BEO (cinnamonaldehyde and thymol) | 100 mg/kg   | Broilers | No effect on FBW, ADG, FCR, CT and ileal ND. Reduction of rectal *E. coli and Clostridium perfringens* counts. | [33]      |
| BEO (garlic, sage, echinacea, thyme, oregano) | 1 g/kg       | Broilers | No effect on FBW, ADG, FCR, CT and ileal ND. Reduction of rectal *E. coli and Clostridium perfringens* counts. | [34]      |
| BEO (oregano, laurel leaf and lavender) | 50 mg/kg    | Broilers | No effect on BWG, FI, FCR, intestinal length and caecal weight. Reduction of faecal *Eimeria* oocyst output. | [35]      |
| BEO (oregano, cinnamonaldehyde, carvacol, yucca extract) | 250 mg/kg | Broilers | No effect on BWG and FI. Improvement of FCR, ATTD of DM and gross energy. Reduction of lesion score and *C. perfringens* and *E. coli* intestinal counts. | [36]      |
| BEO (Agrimonia eupatoria, Echinacea angustifolia, Ribes nigrum and Cinchona succirubra extracts) | 0.5-1.0 g/kg | Broilers | No effect on caecal lesion score. Improvement of BWG and FCR. Reduction of *Eimeria tenella* oocysts count and bloody diarrhea intensity. | [37]      |
| BEO (oregano, anise and citrus peel) | 125 mg/kg   | Broilers | No effect on BWG, FI, intestinal pH values, caecal TVFA levels and total ileum microbiota counts. Improvement of FCR. Reduction of ileum ammonia concentration. | [38]      |
| BEO (clove and cinnamon) | 100 mg/kg   | Broilers | No effect on FBW, ADG, FCR and CT.                                      | [39]      |
| BEO (capsaicin, cinnamonaldehyde, carvacrol) | 150–300 mg/kg | Broilers | No effect on FBW, ADG, FCR, CT and ileal ND. Reduction of rectal *E. coli and Clostridium perfringens* counts. | [40]      |
| BEO (capsaicin, cinnamonaldehyde, carvacrol) | 100 mg/kg   | Broilers | No effect on FBW, CT and ileal ND. Improvement of FCR. Increase of LAB counts and lipase activity in pancreas and intestine wall. Reduction of intestinal *E. coli* and *Clostridium perfringens* counts. | [22]      |
| BEO (capsicum oleoresin, cinnamonaldehyde, carvacrol) | 100 mg/kg   | Broilers | No effect on FBW. Improvement of FCR. Increase of mucus secretion intensity and accumulation inside cells of the gastrointestinal mucosa. Reduction of intestinal *E. coli* and *Clostridium perfringens* counts. | [19]      |
| BEO (thymol, eugenol and piperine) | 50 mg/kg    | Broilers | No effect on FBW, ADG, FI, FCR and LAB counts. Increase of pancreatic trypsin, pancreatic alpha-amylose and intestinal maltase activity. Reduction of *E. coli* counts in ileo-caecal digesta. | [21]      |
| Essential Oil or Component | Level       | Animal  | Effects                                                                 | Reference |
|---------------------------|------------|---------|-------------------------------------------------------------------------|-----------|
| BEO (basil, caraway, lemon, oregano, sage, tea and thyme) | 30 mg/kg   | Broilers | No effect on FI. Improvement of FBW, ADG, FCR and CT. Increase of caecal villus surface area. | [41]      |
| BEO (carvacrol, 1,8-cineole, camphor, thymol, oregano EO, laurel leaf EO and lavender EO) | 75 mg/kg   | Broilers | No effect on CT. Negative effect on FCR. Reduction of FBW, ADG, FI, caecum weight, intestinal length and faecal Eimeria spp. oocyst excretion. | [42]      |
| BEO1 (thymol, eugenol and piperine) or BEO2 (thymol, carvacrol, eugenol and piperine) | 100 mg/kg  | Broilers | Reduction of intestinal Clostridium perfringens counts. | [43]      |
| BEO (thymol, eugenol and piperine) | 100 mg/kg  | Broilers | No effect on BWG, FI, FCR, lesion scores and Eimeria sp. oocyst counts. Modulation of intestinal microbial communities. | [44,45]   |
| BEO (thymol, eugenol and piperine) | 300 mg/kg  | Broilers | No effect on BWG, FI, FCR and caecal microbial population. Slight modulation of intestinal microbial population. | [46]      |
| BEO (thymol, cinnamaldehyde) | 15 + 5 mg/kg | Broilers | No effect on FI, FCR and caecal bacterial (LAB, E. coli, Clostridium perfringens) counts. Increase of BWG. | [47]      |
| Capsaicin | 5–20 mg/kg | Broilers | Reduction of Salmonella Typhimurium counts. | [48]      |
| Grape Seed Proanthocyanidin Extract | 12 mg/kg | Broilers | Increase of BWG. Reduction of lesion scores. Restoration of the antioxidant/oxidant system balance after the parasite infection. | [49]      |
| Mushroom (Lentinus edodes or Tremella fuciformis) or herb (Astragalus membranaceus Radix) polysaccharide extracts | 2 g/kg | Broilers | No effect on BWG, FI, FCR. Increase of bifidobacteria and LAB counts and reduction of Bacteroides spp. and E. coli counts. | [50,51]   |
| Mushroom (Lentinus edodes) extract | 100 g/L water | Broilers | No effect on BWG, FI, FCR and CT. Promotion of bifidobacteria growth. | [52]      |
| Oregano EO | 300 mg/kg   | Broilers | No effect on FBW and CT. Improvement of FCR. Reduction of Fl and excreta oocyst counts. | [53]      |
| Oregano EO | 250–500 g/kg | Broilers | No effect on BWG, FI, FCR, digesta pH, weight and height of the intestinal parts and lipase and amylase activity. Increase of chymotrypsin activity and CPD. | [54]      |
| Oregano EO | 12–24 mg/kg | Broilers | No effect on BWG and FI. Improvement of FCR, intestinal morphological development and enzymatic activities (amylase). | [55]      |
| Oregano EO | 300 mg/kg   | Broilers | No effect on FI. Improvement of BWG and FCR. Reduction of lesion score and Eimeria tenella oocyst counts. | [56]      |
| Oregano EO | 5–10 µL/kg  | Broilers | Intense bacteridical action against lactobacilli and E. coli in faecal samples. | [57]      |
| Oregano or garlic EO | 300 mg/kg  | Broilers | No effect on FI, FCR, CT, ileal Streptococcus, LAB and CB counts. Oregano EO results in reduced FBW (no effect of garlic EO). Reduction of ileal Clostridium perfringens counts. | [58]      |
| Oregano EO | 500 mg/kg   | Broilers | Improvement of BWG and FCR. Reduction of coccidiosis lesion scores and faecal oocyst counts. | [59]      |
| Oregano EO | 600 mg/kg   | Broilers | Improvement of BWG and FCR. No effect on LAB counts, but decrease of E. coli counts. | [60]      |
| Essential Oil or Component | Level |
|---------------------------|-------|
| Oregano EO                | 0.5–1 g/kg |
| Oregano EO                | 300–600 mg/kg |
| Oregano EO                | 330 mg/kg |
| Peppermint EO             | 400 mg/kg |
| Renga renga lily or Acacia extract | 10 g/kg |
| Sophora flavescens extract | 6–30 g/L water |
| Thyme EO                  | 1 g/kg |
| Thyme EO                  | 0.5 g/kg |
| Thyme EO                  | 60 mg/kg |
| Tulbaghia violacea extract | 35 mg/kg |
| Thyme EO                  | 1 g/kg |
| Thyme EO                  | 1 g/kg |
| Thyme EO                  | 0.5 g/kg |
| Thyme EO                  | 1 g/kg |
| Artemisia annua L. extract | 2.5–5.0 mL/kg |
| Thyme EO                  | 0.5 g/kg |
| BEO (thymol, eugenol, piperine) | 30 mg/kg |
| BEO (thyme, rosemary, oregano) | 100 mg/kg |
| BEO (buckwheat, thyme, curcuma, black pepper and ginger) | 250–500 mg/kg |

**Table 1. Cont.**

| Animal | Effects | Reference |
|--------|---------|-----------|
| Broilers | No effect on intestinal villous height and crypt depth. Reduction of *Eimeria tenella* oocyst counts. | [61] |
| Broilers | No effect on BWG and FI. Improvement of FCR. Reduction of lesion score and faecal *Eimeria* sp. oocyst counts. | [62] |
| Broilers | No effect on FCR and lesion scores. Increase of FBW, FI and reduction of caecal *Clostridium perfringens* counts. | [63] |
| Broilers | No effect on BW, ADG, FI and FCR, faecal DMD and CPD and intestinal morphology. | [64] |
| Broilers | No effect on BWG, FI, FCR, intestinal morphology and ileal CB and *Clostridium perfringens* counts. Increase of ileal LAB counts. | [65] |
| Broilers | Increase of BWG. Reduction of lesion scores and faecal *Eimeria tenella* oocyst counts. | [66] |
| Broilers | No effect on FCR, AME, ATTD and intestinal microflora populations. Increase of BWG and FI. | [67] |
| Broilers | No effect on ADG, FI, FCR, DMD, CPD and TVFA. Reduction of caecal isobutyric+ isovaleric levels. | [68] |
| Broilers | No effect on caecal and large-intestinal bacterial counts. Improvement of intestinal barrier integrity and antioxidant status. | [69] |
| Broilers | No effect on FBW, ADG, FI, FCR, ileal CPD and activity of digestive enzymes in intestinal contents and pancreatic tissue. | [70] |
| Broilers | No effect on BWG. Improvement of FCR and reduction of *Eimeria* sp. oocyst counts. | [71] |
| Quails | No effect on FI, intestinal parameters and CT. Improvement of BWG and FCR. Reduction of abdominal fat levels. | [72] |
| Quails | No effect on FI and FCR. Increase of FBW, carcass weight ileal LAB counts and reduction of ileal *E. coli* counts. | [73] |
| Rabbits | Reduction of faecal oocyst and caecal TBC. Improvement of FCR and RGR. | [74] |
| Rabbits | No effect on faecal and caecal bacterial counts. Improvement of intestinal integrity and antioxidant status. | [75] |
| Turkeys | Improvement BWG, FCR and antioxidant status. Increase of caecal LAB counts and reduction of caecal CB counts. | [76] |
| Grower-finisher pigs | Improvement of ADG, FCR, GED, CPD (grower period) and ADG (grower-finisher period). Reduction of ammonia excretion. | [77] |
| Grower pigs | No effect on FCR. Increase of BWG, FI and reduction of faecal noxious gas (ammonia and hydrogen sulide) content. | [78] |
Table 1. Cont.

| Essential Oil or Component | Level          | Animal          | Effects                                                                                      | Reference |
|----------------------------|----------------|-----------------|----------------------------------------------------------------------------------------------|-----------|
| BEO (oregano, anise, orange peel, and chicory EOs) | 125 mg/kg      | Weaner pigs     | No effect on ADG, FI, FCR. Improvement of DM and N digestibility. Reduction of faecal *Salmonella typhimurium* and *E. coli* counts. Increase of faecal Lactobacillus spp. count. | [79]      |
| BEO (thymol and cinnamaldehyde) | 0.1–0.15 mg/kg | Weaner pigs     | Improvement of ADG and FCR. Increase of FI and faecal LAB counts. Reduction of diarrhea occurrence and faecal *E. coli* counts. | [80]      |
| BEO (thymol and cinnamaldehyde) | 100 mg/kg      | Weaner pigs     | Improvement of ADG, DMD, CPD and faecal score. Increase of villus height to crypt depth ratio in the jejunum, caecal LAB counts and reduction of caecal and rectal *E. coli* counts. | [81]      |
| BEO (peppermint, anise and clove) | 300 mg/kg     | Weaner pigs     | No effect on BWG, FI and gastrointestinal microbiota. Improvement of FCR, CPD and amino acids digestibility. | [82]      |
| BEO (oregano, cinnamon and Mexican pepper) | 150–300 mg/kg | Weaner pigs     | No effect on BWG, FI, FCR and ATTD. Increase of gastric retention time and pH, lactobacilli:enterobacteria ratio and decrease of ileum total microbial count. | [83]      |
| BEO (oregano, cinnamon and Mexican pepper) | 300 mg/kg      | Weaner pigs     | No effect on BWG, FI, FCR, ATTD, intestinal pH and gastrointestinal morphology.                 | [84]      |
| BEO (oregano, cinnamon and Mexican pepper) | 200 mg/kg      | Weaner pigs     | No effect on BWG, FI and FCR. Increase of ileal lactobacilli:enterobacteria ratio and decrease of TVFA production in the cecum. | [85]      |
| BEO (carvacrol and thymol) | 500–1500 mg/kg | Weaner pigs     | No effect on BWG, FI, FCR, praecaecal digestibility, enzyme activities, faecal microbial counts and intestinal microflora. | [86,87]  |
| BEO (cinnamon, thyme and oregano extract) | 750 mg/kg      | Weaner pigs     | No effect on BWG, FI, FCR, intestinal morphology and LAB counts. Reduction of CB counts.         | [88]      |
| BEO (buckwheat, thyme, curcuma, black pepper and ginger) | 250 mg/kg   | Weaner pigs     | No effect on ADG, FI, FCR and caecal LAB counts. Improvement of CPD and DMD and reduction of faecal *E. coli* counts. | [89]      |
| BEO (thymol, cinnamaldehyde) | 250 mg/kg      | Weaner pigs     | No effect on FI, caecal *E. coli* and LAB counts. Improvement of ADG, FCR, DMD, CPD and GED. Increase of villus height (jejenum) and reduction of rectal *E. coli* counts. | [90]      |
| Phytoncide                   | 2 g/kg         | Weaner pigs     | No effect on ADG, FI, FCR, faecal *E. coli* counts and diarrhea scores. Improvement of DMD and increase of faecal LAB counts. | [91]      |
| Powder of medicinal herbs (Panax ginseng, Dioscoreaceae opposite, Atractylodes macrocephala, Glycyrrhiza uralensis, Ziziphus jujube and Platycodon grandiflorum) | 1–3 g/kg    | Weaner pigs     | No effect on FI, BWG and FCR. Increase of DMD, CPD, GED, villous height and ileal and caecal LAB counts and reduction of caecal CB counts. | [92]      |

ADG: average daily gain; AME: apparent metabolisable energy; ATTD: apparent total tract digestibility; BEO: blend of essential oils; BWG: body weight gain; CB: coliform bacteria; CT: carcass traits; CPD: crude protein digestibility; DM: dry matter; DMD: dry matter digestibility; DE: digestive tract; EO: essential oil; FBW: final body weight; FCR: feed conversion ratio; FI: feed intake; ND: nutrients digestibility; GP: growth performance; LAB: lactic acid bacteria; RGR: relative growth rate; TBC: total bacterial counts; TVFA: total volatile fatty acids.
3.2. Effects of EOs on Rumen Fermentation

Ruminal microbial activity plays an important role in the synthesis of high-quality protein for ruminants. However, microbial fermentation could lead to significant energy and protein losses as methane and ammonia that are significant environmental pollutants. Essential oils could be used to manipulate ruminal metabolism and selectively inhibit rumen methanogenesis due to their antimicrobial properties. Methane (CH$_4$) is a potent greenhouse gas with a global warming potential 21 times that of carbon dioxide (CO$_2$) [93]. At the same time, enteric methane losses represent 2–12% of gross energy intake in ruminants depending on diet composition and feed intake [94]. It can be therefore concluded that the reduction of CH$_4$ emissions through the application of EOs is beneficial both for the animals (improved feed efficiency and productivity) and for the environment (mitigation of greenhouse effects) [95]. Essential oils could also positively influence protein metabolism and reduce rumen ammonia levels and lead to a more efficient utilization of dietary nitrogen by inhibiting deamination, i.e., the breakdown of amino acids to NH$_3$, possibly through the selective limitation of the activity of a specific group of bacteria within the rumen, the “hyper-ammonia-producing bacteria” (Prevotella spp., Ruminobacter amylophilus, etc.) at the level of attachment and colonization [96,97].

Several EOs (thyme, oregano, cinnamon, garlic, etc.) have already been used with the intention to reduce CH$_4$ production. At low doses, ruminal fermentation is not affected by essential oils, whereas at high doses these compounds inhibit the target microbial species as well as most rumen microbes [98]. As it has been demonstrated in in vitro studies, although the effects of EOs are diet (forage:concentrate ratio), pH (more intense action at low pH values) and time (adaptation period) dependent, mitigation of methane emissions occurs at high doses (>300 mg/L of culture fluid) and is frequently associated with a decrease in total volatile fatty acids concentration and feed digestion. As a result, although EOs in high doses could exert positive in vitro effects on rumen fermentation, these doses result in negative implications on feed palatability, digestion and animal productivity when applied in vivo. At the same time, the levels of EOs that have elicited favorable fermentation responses in vitro are far too high for in vivo application due to their possible toxic effects and high cost [99]. As shown in Table 2, no effects on feed intake, average daily gain and total volatile fatty acids concentrations and rates (i.e., acetate to propionate ratio) are generally observed after the dietary supplementation of ruminants with EOs or their main components as single compounds or mixtures. Since no significant differences in feed intake and rumen characteristics are found, it can be concluded that generally no effects on rumen methanogenesis are induced. A possible explanation is the ability of rumen microbes to adapt and degrade EOs components [95].
Table 2. Effects of essential oils or their components on rumen characteristics and performance parameters.

| Essential Oil or Component | Level | Animal | Effects | Reference |
|----------------------------|-------|--------|---------|-----------|
| Eugenol                    | 50 mg/kg DM | Dairy cows | No effect on DMI, ND, RP and MY. | [100] |
| Cinnamaldehyde and eugenol | 85 & 140 mg/day, respectively | Dairy cows | No effect on DMI, FB, VFA concentration, NH$_3$, A:P ratio, ruminal pH, ND and MY. | [101] |
| Cinnamaldehyde and eugenol | 1.7 & 2.8 g/day, respectively | Dairy cows | No effect on DMI, FB, VFA concentration, NH$_3$, A:P ratio, ruminal pH, ND and MY. | [101] |
| Garlic EO                  | 5 g/day | Dairy cows | No effect on DMI, FB, VFA concentration, NH$_3$, A:P ratio, ruminal pH, ND and MY. Improvement of FD in rumen. | [102] |
| Juniper berry EO           | 2 g/day | Dairy cows | No effect on DMI, ruminal pH, VFA concentration, NH$_3$, A:P ratio, ruminal pH, ND and MY. Improvement of FD in rumen. | [102] |
| Oregano leaves             | 250, 500 or 750 g/day | Dairy cows | No effect on ND, VFA concentration, ruminal pH and MY. Improvement of FD in rumen. | [103] |
| Oregano leaves             | 500 g/day | Dairy cows | No effect on DMI, ND, VFA concentration, ruminal pH and MY. Improvement of FD in rumen. | [104] |
| MEO                        | 2 g/day | Dairy cows | No effect on FI, ND, VFA concentration and MY. Increase of ruminal pH. | [105] |
| MEO                        | 750 mg/day | Dairy cows | No effect on FI, ND, RP and MY. Increase of ruminal pH. | [106] |
| MEO                        | 1.2 g/day | Dairy cows | No effect on BW, BCS and VFA concentration. Increase of DMI and MY. | [107] |
| Blend of oregano, cinnamon, thyme and orange peel EOs | 0.32, 0.64 or 0.96 g/day | Dairy cows | No effect on DMI, ND and MY. | [108] |
| Mixture of eugenol, geranyl acetate and coriander oil | 1 g/day | Dairy cows | No effect on DMI, ND and MY. Decrease of BCS. | [109] |
| MEO                        | 1 g/day | Steers | No effect on DMI, ADG, CC, ND, VFA concentration and ruminal pH. | [110] |
| Cinnamon EO                | 5 g/day | Calves | No effect on DMI, ADG, VFA concentration and ruminal pH. Decrease of A:P ratio. | [111] |
| Thyme EO                   | 5 g/day | Calves | No effect on DMI, ADG, VFA concentration and ruminal pH. Decrease of A:P ratio. | [111] |
| Anise extract              | 500 mg/day | Beef heifers | Reduction of VFA concentration and RP (A:P ratio, NH$_3$). | [112] |
| Cashew and castor EOs      | 3 g/day | Bulls | No effect on DMI, ND and CC. Improved ADG, final BW and FE. | [113] |
| Essential Oil or Component | Level | Animal       | Effects                                                                 | Reference |
|----------------------------|-------|--------------|-------------------------------------------------------------------------|-----------|
| Coconut oil                | 7%    | Swamp buffaloes | Negative effect on DMI, VFA concentration, A:P ratio and MP. No effect on ruminal pH, NH₃ and ND. | [114]     |
| Eucalyptus EO              | 2 mL/day | Swamp buffaloes | No effect on DMI, ND, ruminal pH, VFA concentration. Decrease of MP. | [115]     |
| Carvacrol                  | 200 mg/kg DM | Lambs | Reduction of ruminal pH and increase of VFA concentration. No effect on other RP (A:P ratio, NH₃), DMI, AVG, CC and MC. | [116]     |
| Cinnamaldehyde             | 200 mg/kg DM | Lambs | Reduction of ruminal pH, increase of VFA concentration. No effect on other RP (A:P ratio, NH₃) DMI, AVG, CC and MC. | [116]     |
| Cinnamaldehyde             | 200 mg/kg DM | Lambs | No effect on RP, DMI, CC and MC. Positive effect on AVG. | [117]     |
| Coconut oil                | 25, 50 or 75 g/kg of CF | Lambs | No effect on ADG and CC. Decrease of DMI. | [118]     |
| Garlic EO                  | 200 mg/kg DM | Lambs | No effect on RP, DMI, AVG, CC and MC. | [117]     |
| Juniper berry EO           | 200 mg/kg DM | Lambs | No effect on RP, DMI, CC and MC. Positive effect on AVG. | [117]     |
| MEO                        | 50, 100 or 150 mg/kg DM | Dairy ewes | No effect on DMI and ruminal pH. Positive dose-dependent effect on MY. | [119]     |
| Garlic EO                  | 5 g/kg DM | Sheep       | No effect on DMI and MP. | [120]     |
| Mixture of clove, oregano, cinnamon, and lemon EOs | 1 g/day | Sheep | No effect on DMI, ND and ruminal pH. Decrease of VFA concentration, A:P ratio and RPD. | [121]     |
| Mixture of eugenol, carvacrol, citral and cinnamaldehyde | 1 g/day | Sheep | No effect on DMI, ND and ruminal pH. Decrease of VFA concentration, A:P ratio and RPD. | [121]     |
| Resveratrol                | 0.25 g/day | Sheep | No effect on DMI, ruminal pH, NH₃ and VFA concentration. Positive effect on ND. Decrease of MP and A:P ratio. | [122]     |
| Mixture of linalool, p-cymene, alpha-pinene & beta-pinene | 0.043 or 0.43 g/kg DM | Dairy goats | No effect on DMI, ND, VFA concentration, A:P ratio and MY. | [123]     |

ADG: average daily gain, A:P ratio: acetate: propionate ratio, BCS: body condition score, BW: body weight, CC: carcass characteristics, CF: concentrated feed, DM: dry matter, DMI: dry matter intake, FB: feeding behavior, FD: feed digestibility, FE: feed efficiency, FI: feed intake, MC: meat characteristics, MEO: EOs components mixture (thymol, eugenol, vanillin, guaiacol and limonene), MP: methane production, MY: milk yield, ND: nutrients digestibility, NH₃: ammonia, RP: rumen parameters, RPD: ruminal protein digestibility, VFA: volatile fatty acids.
4. Antioxidant Effects of EOs

Oxidation of lipids and free radicals’ production are natural processes that destroy the membrane structure, disturb transport processes and cause losses in the function of the cell organelles. Lipids and especially phospholipids present in cell membranes are particularly susceptible to oxidative damage that is positively correlated with the degree of unsaturation of the fatty acids. Polyunsaturated fatty acids (PUFAs) are responsible for the maintenance of physiologically important cell membrane properties including fluidity and permeability. The peroxy radicals react with PUFAs and form hydroperoxides (ROOH), which later decompose to produce the volatile non-radical aromatic compounds (aldehydes, alkanes, conjugated dienes, etc.) that adversely affect lipids, pigments, proteins, carbohydrates vitamins and the overall quality of animal products by causing loss of nutritive value and limiting shelf-life [124].

Although synthetic antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), gallates, etc.) are traditionally used with the intention to delay, retard or prevent the negative effects of lipid peroxidation by scavenging chain-carrying peroxyl radicals or diminishing the formation of initiating lipid radicals, the demand for natural antioxidants has recently been increased. During the last decades interest in employing antioxidants from natural sources to increase the shelf life of foods is considerably enhanced due to consumer preference for natural occurring ingredients and concerns about the possible toxic effects of synthetic antioxidants.

Animal diet plays a crucial role in the inhibition of free radicals production in the organism and the derived products. The nutritional prevention of oxidizing stress and its implications suggests the optimization of the antioxidant levels in the feed. As illustrated in Table 3, quality of animal products is generally improved after the incorporation of oregano or rosemary EO into the diets of animals.

EOs are rich sources of natural antioxidants, such as the phenolic compounds and due their high redox properties and chemical structure have the ability to neutralize free radicals, chelate transitional metals and quench singlet and triplet oxygen by delocalization or decomposition of peroxides [125,126]. As a result, they affect lipid metabolism in animal tissues by exerting beneficial effects on the antioxidative enzymes activity (superoxide dismutase, catalase and glutathione peroxidase) and by preventing the production of reactive oxygen species and off-flavors deriving from the oxidation of polyunsaturated fatty acids [127]. However, depending on type and dosage, EOs can act as prooxidants by disrupting the integrity of cell membrane and organelles, with further cytotoxic effects on living cells [128]. Dietary supplementation with EOs is therefore a simple and convenient strategy to uniformly introduce natural antioxidants into phospholipid membranes, where they may effectively inhibit the oxidative reactions by preventing the formation of radicals, by scavenging them, or by promoting their decomposition at their localized sites and appears as a more effective way of retarding lipid oxidation of animal products compared to post mortem addition [129,130].
Table 3. Dietary supplementation of animal diets with the two most common essential oils derived from the Greek flora (oregano and rosemary) and effects on the quality of the derived products.

| Essential Oil | Major Antioxidant Compounds                                      | Level          | Product      | Effect | Reference |
|---------------|------------------------------------------------------------------|----------------|--------------|--------|-----------|
| Oregano       | p-cymene, gamma-terpinene, beta-Caryophyllene, borneol, carvacrol, thymol | 100–200 mg/kg | Egg          | +      | [131]     |
|               |                                                                  | 100 mg/kg      | Chicken Meat | +      | [132]     |
|               |                                                                  | 50–100 mg/kg   | Chicken Meat | +      | [133–135] |
|               |                                                                  | 300 mg/kg      | Chicken Meat | NS     | [136]     |
|               |                                                                  | 500 mg/kg      | Chicken Meat | +      | [137]     |
|               |                                                                  | 1.0 mL/kg      | Lamb Meat    | +      | [138]     |
|               |                                                                  | 0.25–1.0 mL/kg | Pork Meat    | NS     | [139]     |
|               |                                                                  | 500 mg/kg      | Pork Meat    | NS     | [140]     |
|               |                                                                  | 100–200 mg/kg  | Rabbit Meat  | +      | [141]     |
|               |                                                                  | 100 mg/kg      | Turkey Meat  | +      | [14,142]  |
|               |                                                                  | 200 mg/kg      | Turkey Meat  | +      | [129,143] |
|               |                                                                  | 1 mL/kg        | Sheep Milk   | NS     | [144]     |
| Rosemary      | Carnosic acid, carnosol, rosmanol, rosmarinic acid               | 200 mg/kg      | Quail egg    | +      | [145]     |
|               |                                                                  | 1.0 g/kg       | Beef         | +      | [146]     |
|               |                                                                  | 400 mg/kg      | Lamb Meat    | NS     | [147]     |
|               |                                                                  | 0.6 g/kg       | Lamb Meat    | NS     | [148,149] |
|               |                                                                  | 0.4 g/kg       | Lamb Meat    | +      | [150,151] |
|               |                                                                  | 500 mg/kg      | Chicken Meat | +      | [152]     |
|               |                                                                  | 100–200 mg/kg  | Chicken Meat | +      | [153]     |
|               |                                                                  | 40 mg/kg       | Pork Meat    | +      | [154]     |
|               |                                                                  | 0.6–1.2 g/day  | Sheep Milk   | +      | [155]     |

+: positive effect, NS: no effect.
5. Conclusions

The increasing pressure on the livestock industry to stop the use of antibiotics as growth promoters has initiated new research to find safe and efficient alternatives. The wide range of bioactive compounds included in the essential oils could boost their use as multifunctional feed supplements for animals. EOs contain several components that have the potential to positively manipulate gut microbiota and rumen fermentation, inhibit pathogenic bacterial growth, prevent tissue oxidation and, as a result, improve growth performance and products’ quality of livestock. However, their effectiveness in animal production has not yet been proven to be consistent and conclusive and some issues need to be addressed before their commercial application. For example, an optimal dose of essential oils application, depending on their type, should be addressed, since their use at high concentrations could induce cytotoxic effects on living cells. Although EOs are generally recognized as safe (GRAS) for use in the food and feed industry and the accumulation of their components in the body is unlikely due to their rapid elimination (glucuronides by the kidneys) or exhalation (carbon dioxide), no analytical methods suitable for their identification and quantification (traceability, toxicity, possible residues, etc.) have yet been developed. At the same time, the beneficial effects of EOs dietary supplementation should be such as to justify the additional cost of their application. Finally, despite the fact that the understanding of EOs mode of action is a prerequisite for their regular application in animal production, our knowledge regarding their activities in animal (as well as in human) organism is still rather limited and there is a strong need for information regarding their absorption, distribution, metabolism and excretion. A further clarification of the above inquiries would therefore be beneficial for establishing EOs regular application in animal production.

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