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Review

Essential Oil Supplementation in Small Ruminants: A Review on Their Possible Role in Rumen Fermentation, Microbiota, and Animal Production

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Abstract: Essential oils are bioactive compounds, originating from the secondary metabolism of plants, recognized for their ability to modify rumen fermentation, gut health, and to function as antioxidant molecules in small ruminants. Indeed, small ruminant-derived products, such as milk, dairy, and meat can benefit from the utilization of essential oils, that have demonstrated antimicrobial, antioxidant and anti-inflammatory affects, in the animals' diet. This review reports on the findings that demonstrates the possible role of essential oils in controlling greenhouse gas emissions from ruminants through the modulation of ruminal microbial populations, in sustaining animal health and welfare by affecting the gut microbiota, and in ameliorating animals' products through enhancement of their nutritional composition from a human diet perspective. However, the current review highlighting the inconclusive findings related to the use of essential oils in small ruminant nutrition, supports the need of further studies to better understand the administration of how essential oils and to explore their specific actions at the molecular level.

Keywords: small ruminants; essential oils; environment; animal production

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1. Introduction

Plant-derived essential oils (EOs) are a group of organic compounds obtained from the secondary metabolism of plants responsible for the odor of plants and spices, which often exhibit antimicrobial activity, thus showing the ability to control plant diseases [1]. The first experiments on EOs started from the observations that grazing ruminants avoided some plants not only for their organoleptic properties, but also for their negative effects on rumen microbial fermentation and nutrient digestion [2,3]. The bioactive compounds contained in EOs are recognized for their ability to modify rumen and gut functioning by favoring the digestive processes, to act on bacterial growth, and to control oxidation. Studies on the effects of EO on rumen microbiota were conducted until the beginning of 1970s, when the intensification of the use of ionophore antibiotics as growth promoters in animal feeding delayed further research on the use of EO.

In Europe, the use of ionophore antibiotics as feed additives and growth promoters in animal feeding has been banned since 2006. Moreover, the Farm to Fork strategy, the new approach of the European Green Deal, aims at driving the European food systems toward a more sustainable route by reducing global greenhouse gas (GHG) emissions and the loss of biodiversity, ensuring food security, and providing access to sufficient, safe, nutritious, and sustainable food. From this point of view, the use of EO in ruminants' diet may be a suitable instrument to optimize rumen fermentation and improve efficiency of energy and nutrient utilization; the ability of EO to modify the rumen environment by enhancing or inhibiting specific microbial populations is a crucial point for ruminant nutritionist to control and reduce GHG emissions [4]. It has been summarized that a proper optimization of diet formulations and the utilization of EO as feed additives may also contribute to sustain the gut microbiota health and to enhance ruminant immune responses, therefore resulting in sustainable and healthier foods for human diet [5]. Consumers' acceptance has a significant impact on the use of antibiotics in animal feed, and as a consequence, feed additive with the ability to enforce ruminant immune system in order to reduce the use of antibiotics could strategically increase animal-based food acceptability. Finally, the use of EOs in ruminants' diet may improve their performance and welfare, and reduce their impact on the environment, ensuring more sustainable animal-based foods.

The majority of studies on the effects of EO in animal diet mainly address the non-ruminant species or dairy cows; as a result, data on the efficacy of EO fed to small ruminants is very limited, and the results obtained are not conclusive. Therefore, the objective of the present paper is to review the current knowledge on the use of EO in small ruminants' diet, and to report on their effects on rumen microbial fermentation and microbiota changes, gut microbiota and milk quality.

2. Effects of Essential Oils on Rumen Microbiota

Rumen and gut microbiota can be modified and modulated by the activity of EO. Such effects usually comprise the regulation of fermentation processes which could also lead to a reduction in methane production. Considering that methane is a greenhouse gas and it represents a loss of gross energy from feed, any reduction in methane from ruminal fermentation represents an increase in feed efficiency and reduces ruminant farming's environmental impact. However, considering the experiments performed to verify the effects of EO administration in animal science, very few studies dealt with the effects of EO on rumen microbiota and even less in small ruminants, whereas a few studies concentrated on the effects of EO administration in the diet on gut microbiota in pigs and poultry. The chemical components of EOs that exhibit antibacterial activities and antioxidant properties, and in general show biological properties, are reported in Table 1.

Table 1. Essential oils and their active components with demonstrated biological components.

Essential Oil	Commercial Name	Active Components	Reference
Allium sativum	Garlic	Allicin, diallyl sulfite	[6]
Anethum graveolens	Dill	Limonene, carvone	[7]
Artemisia herba alba	White wormwood	Limonene, fenchol, α-thujone, camphor, nordavanone	[8]
Capsicum annum	Paprika	Capsaicin	[7]
Cinnamomum cassia	Cassia	Cinnamaldehyde	[9–11]
Coriandrum sativum	Coriander	Linalool	[12,13]
Juniperus oxycedrus	Juniper	Cadinene, pinene	[14]
Laurus nobilis	Laurel	1,8 Cineole, borneol	[15]
Melaleuca alternifolia	Tea tree	Terpinen-4-ol	[16,17]
Nigella sativa	Black cumin	Thymoquinone, Carvacrol, ρ-cymene	[18]
Origanum vulgare	Oregano	Carvacrol, thymol, γ-terpinene	[19–27]
Pelargonium graveolens	Rose geranium	Citronellol, Geraniol, linalool, citronelleyl formate, ρ-menthone	[28]
Pimpinella anisum	Anise	Anethol	[7]
Rosmarinus officinalis	Rosemary	1,8-Cineole, Camphor, α-Pinene, bornyl acetate	[9,11,23,29,30]
Salvia officinalis	Sage	Camphor, α-Pinene, β-Pinene, 1,8 Cineole, α-Thujone	[22]
Syzygium aromaticum	Clove	Eugenol	[9,11]
Thymus vulgaris	Thyme	Thymol, carvacrol, δ-terpinene, ρ-cymene	[9,10,23,31–35]
Zingiber officinale	Ginger	Zingiberene, zingerone	[16]

Adapted from Calsamiglia et al. [36] and Nehme et al. [37].

Essential oils derived from thyme, fennel, ginger, black seed, and Eucalyptus oil added to the basal diet altered rumen fermentation and, by using in vitro gas production techniques, significant differences regarding fermentation gas production and methane formation were found; methane efficiency increased from 53% to 166% as compared with the control [38]. The highest dosage of black seed inhibited methane efficiency in about 62% of EOs. Among 100 EOs and plants tested from North America, *Anethum graveolens* (dill weed), *Lavandula latifolia* and *Ocimum basilicum*, and one plant sample of *Origanum vulgare* were found to reduce methane production in in vitro experiments [39]. In sheep oregano, EO supplementation can modify in vitro ruminal fermentation by altering volatile fatty acid (VFA) concentrations and reducing methane emissions with an extensive alteration of the ruminal bacterial community [40]. Such alteration of rumen bacteria diversity by oregano EO can improve feed digestibility and ruminal fermentation characteristics, and shift fermentation to reduce molar concentrations of acetate and butyrate and increase propionate. The optimal feeding rate for mature ruminants, suggested by Zhou et al. [40], could be approximately 52 mg/L. It was reported that thymol, the main component of oregano EO, was able to affect the integrity of the cell membrane of pure culture of *Streptococcus bovis* and *Selenomonas ruminantium*, thus reducing their uptake of glucose [41]. Thymol can reduce methane and lactate concentrations, with an increase in the acetate-to-propionate ratio at moderate doses (100 to 400 mg/L), and an inhibition of microbial metabolism at higher doses. Thymol is particularly effective as antimicrobials because of its small molecular weight and the presence of a hydroxyl group in its phenolic structure, which facilitates the access to the cell membrane through the pores of the external wall [42–44]. Also, both garlic oil and diallyl disulfide contained in *Allium sativum* were found to decrease in vitro methane and total VFA when applied at a concentration of 300 ppm, and to have no effects when tested at 30 ppm in vitro [45].

Results from in vivo studies are controversial. Khiaosa-Ard and Zebeli [46] found that in small ruminants and beef cattle, EOs were more effective in decreasing rumen acetate and methane production than in dairy cows. When sheep were administrated with ropadiar, an essential oil of the oregano plant, at a dose of 250 mg/d, the average methane production decreased compared to the control [47]. The authors suggested that methane emission from rumen may be affected by the altered concentrations of NH_3-N and VFA in rumen liquor. Moreover, methane production was positively correlated with the concentrations of NH_3-N, and negatively correlated with total VFA and the proportion of propionate of rumen liquor. In dairy ewes of Chios breed, the administration of a mixture of EO, 100 and 150 mg of EO/kg in a concentrate diet, led to a decrease in rumen urea, reduced the acetate to propionate ratio, increased cellulolytic bacteria numbers, and decreased hyper-ammonia-producing bacteria [48]. It appeared that the ability of EOs to alter methane production could be determined by the inhibition of the activity of rumen methanogenic bacteria such as archea and hyper-ammonia-producing bacteria [49]. EOs from clove, eucalyptus, garlic, oregano, and peppermint cause a decline in the number of rumen archaea and protozoa, as well as in cellulolytic bacteria [50]. This event can be claimed to explain the reduction in not only methane production but also in fiber digestion observed in the presence of EO.

However, the effects of EO on rumen bacteria, protozoa, and fungi can be both an inhibitory effect, with a reduction in rumen microbial abundance, and a stimulatory effect, with an increase in rumen microbial abundance, depending on the type of EO. When *Pistacia atlantica subspecies Kurdica* gum EO, named PAKEO, was tested in an in vivo experiment on sheep, the protozoa population was not affected with PAKEO treatment, while the relative abundance of total bacteria increased by 10%, *Ruminococcus flavefaciens* increased by 62%, and the abundance of rumen methanogens decreased by 32% [51]. On the contrary, Kim et al. [52] found an increase in relative protozoa abundance following the administration of a commercial essential oil mixture as a feed additive, in which the main constituents were cinnamaldehyde, thymol, and eugenol. In particular, the in vitro experiment tested on a culture fermentation batch from heifers with two essential oil mixture concentrations resulted in higher *Selenomonas ruminantium* and *Ruminococcus albus* abundance when using the lower essential oil mixture concentration, and higher *Butyrivibrio fibrisolvens*, fungi and *Ruminococcus flavefaciens* abundance when using the higher essential oil mixture concentration. On the contrary, when oregano leaves supplementation in dairy cow diet was used, decreased levels of *Ruminococcus Flavefaciens* in rumen was observed [53]. In addition to the different effects on rumen microbiota exerted by different EOs, it should be also taken into account that the composition of EO from the same plant species can change according to different harvesting seasons and geographical locations [54]. Moreover, the same EO can influence ruminal microbiota differently in a dose-dependent way. The effects of EO on rumen microbiota seems also to be highly dependent on ruminal pH, as the pH level can influence the dissociated or undissociated status of EO molecules. Some authors suggested that the undissociated hydrophobic form of the active EO molecules is more effective in its antimicrobial activity because it dissolves better in the lipid bilayer of the bacterial membrane, and this is proven at low rumen pH [55,56]. However, increased pH in 24 h in vitro batch cultures of mixed rumen bacteria was found when 400 mg/L of thymol was added, which is the EO contained in *Thymus* and *Origanum* plants; in addition, no effects were reported at lower doses [41]. Such pH increase is associated with the decrease in VFA concentration and methane production in the rumen, and it indicates the inhibition of rumen microbial fermentation. The rumen pH is also linked to diet; consequently, the effects of EO on VFA and methane production is dependent on diet. When thymol oil was added in ruminal fluid of cattle fed a 60:40 forage/concentrate diet at pH 6.4, the acetate/propionate ratio increased, but when thymol was incubated in ruminal fluid of cattle fed a 10:90 forage/concentrate diet at pH 5.5, the acetate/propionate ratio decreased [36].

Moreover, the alteration of ruminal populations with the use of EO can also have an impact on some ruminal microorganisms responsible for bio-hydrogenation of polyunsaturated fatty acids, thus causing a modification of the fatty acid profiles of digesta escaping from rumen and of meat in small ruminants [57]. Therefore, EO supplementation to ruminants should be carefully planned with a high specificity and selection of herbal essence and dose. A proper choice positively impacts the environment, feed efficiency, and milk or meat produced. An inappropriate choice can increase methane production, reduce feed efficiency and even result in possible resistance to EO effects, and thereby worsen ruminal fermentation. The issue of bacterial resistance to EO is a matter of debate; resistant bacteria are those that genetically and casually acquire characteristics, allowing them to survive in the presence of compounds with antimicrobial properties. Based on this, if EO have antimicrobial properties, then bacteria can acquire resistance to EO; however, very few trials investigated bacterial resistance to EO. In their review, Benchar and Greathaed [58] supposed that since EO is a mixture of chemical compounds that exert antimicrobial activity in different ways, bacteria can hardly develop resistance mechanisms [59]. Nevertheless, it has been reported that *Staphylococcus aureus* developed resistance to tea tree (*Melaleuca alternifolia*) essential oil [60,61].

3. Effects of Essential Oils on Gut Microbiota

In their extensive meta-analysis on EO in small ruminants, Dorantes-Iturbide et al. [62] reported that EO administration in the diet can increase feed intake, and hence, suggested the use of EO to increase dry matter intake (DMI) in small ruminants. The role of EO in increasing ruminal absorption of total volatile fatty acid concentration was suggested, which results in an increased average daily gain and a better feed conversion ratio. This role could be even further enhanced by the microencapsulation of EO, as suggested by Nehme et al. [37], which can protect the EO from oxidation during their storage, transport, and processing, and boost their biological effectiveness. Microencapsulation of EO can also help to target the release and action of EO in the intestinal tract and protect them from ruminal alteration. Microencapsulated oregano EO in the diet of Sewa sheep exerted a positive effect on the digestion and absorption of nutrients in the small intestine by regulating intestinal morphology and intestinal microorganisms [63]. Moreover, increased ratio of the villus height to the crypt height in the ileum, duodenum, and jejunum were found, probably as a result of both microencapsulation and choice of the 300 mg/kg oregano EO dose. These results, together with the measured increased richness of some bacterial genera such as Coprococcus (faecal coccus), Clostridium, and Acetobacter, and their enzymes, increased the absorption and utilization of nutrients in the intestinal tract; this could be claimed to explain the improvements in average daily gain. The effects and distribution of microorganisms in the small intestine after the administration of EO was also investigated by Zhang et al. [64] using the 16s rRNA gene sequencing technique Zanthoxylum bungeanum belongs to the Rutaceae family, with abundant presence of flavonoids, among which quercetin, foeniculin, rutin, hyperin, and isoquercitrin are notable. Different doses of Zanthoxylum bungeanum EO (ZB) in the diet of small-tailed Han sheep resulted in the association of differential bacteria, connected with the digestion and absorption of nutrients and the state of intestinal health in the host, in the duodenum, jejunum, and ileum. At the phylum level, the administration of ZB EO resulted in the mergence of differential bacteria Firmicutes, Bacteroidetes, Tenericutes, and Proteobacteria in duodenum. In the jejunum, differential bacteria Firmicutes, Bacteroidetes, Tenericutes, and Proteobacteria emerged, while in the ileum, Firmicutes, Bacteroidetes, and Tenericutes were found. Nevertheless, the same dose of ZB EO resulted in differences in the five most abundant genera of bacteria in the different regions of the small intestine (duodenum, jejunum, and ileum). In particular, the administration of ZB EO at 15 mL/kg reduced the abundance of Bacteroidetes and increased the abundance of Firmicutes, thereby increasing the Firmicutes/Bacteroides (F/B) ratio. Notably, a high F/B ratio sustains microbiota homeostasis and helps to absorb energy in the small intestine [65], endowing Bacteroidetes the ability to increase the utilization of carbohydrates [66], and providing Firmicutes with the role of regulating the digestion of proteins and carbohydrates [67]. Thus, the administration of microencapsulated EO can influence the abundance of differentiated bacteria in the small intestine, and have positive effects on the development of host intestinal epithelial cells, maintenance of microbial and metabolic homeostasis, and control of inflammatory processes. A reduction in inflammatory processes may also be related with the reduced permeability of intestinal epithelial cells, which acts as a barrier to the passage of pathogens and pro-inflammatory mediators; thus, the increase in bacteria that can induce epithelial cell proliferation and tighten intestinal barrier can be considered a favorable event for the regulation of inflammatory processes. In the duodenum, different doses of ZB EO were able to increase both Lachnospiraceae NK3A20 and Clostridium sensu stricto 1, associated with butyrate production, which contributes to host energy supply [68–70]. Butyrate is essential for the development of gastrointestinal epithelial cells, and it helps to accelerate the development and maturation of gut in young ruminants [71]. The regulation of bacterial abundance in the gut was also exerted in calves after the administration of oregano extracts. Their supplementation at 60 mg/kg body weight per day increased the diversity of rumen and jejunum bacterial population, affecting the abundance of both Gram-positive and -negative bacteria in the gut [72]. A decrease in potentially pathogenic genera such Streptococcus, Escherichia, and Clostridium was measured with a concomitant decrease in the population of Bifidobacterium in the jejunum and cecum. The addition of oregano essential oil (52 mg/head/day) in the diet of small-tailed Han × Hu female lambs resulted in an increased average daily gain and a decrease in the feed/gain (F/G) ratio [73]. The authors observed that dietary supplementation with oregano EO promoted the enrichment in intestinal microbiota of Ruminococcus, Bifidobacterium, and Enterococcus, with the increase in the levels of indole-3-acetic acid and indole acetaldehyde. These changes in intestinal microbiota might contribute to an increase in amylase activity, thus improving intestinal barrier function and growth performance. In addition, sheep treated with a dietary supplementation of oregano EO exhibited an increased expression of mucin protein in the jejunal mucosa, and decreased production of serum IL-2 and TNF-β concentration, which are pro-inflammatory cytokines involved in the generation of T cells and cell-mediated immunity. A general suppression of inflammatory signaling pathway expression in the ileum was also attributed to a reduction in the expression of the nuclear factor kappa B p65, Toll-like receptor-4, and pro-inflammatory cytokine IL-6 genes exerted by oregano EO, with the final effect of increasing intestinal integrity.

4. Effects of Essential Oils on Small Ruminant Products

A number of studies on bovines deal with the inclusion of EO in the diet of ruminants in order to improve their milk production. Such an improvement can be considered as the result of the positive effect of EOs on the oxidative status and immune responses of ruminants, which improves animal health and welfare; this mechanism was extensively reviewed in Caroprese et al. [5]. The inclusion of EOs in the diet of small ruminants in order to ameliorate milk and meat production is based on the observation that EOs in general can improve taste and palatability of feed, thereby increasing voluntary intake and weight gain. This expectation is not usually met; however, an increase in the antioxidant activity of both meat and milk is often found.

Muñoz-Cuautle et al. [74] observed that oregano EO, at 0.02% and 0.04%, in the diet of Suffolk × Hampshire male lambs did not result in improved productive performance, backfat thickness and loin eye area; however, the antioxidant activity of lambs' meat was improved. In particular, the addition of 0.02% of oregano EO in the diet of growing lambs preserved the stability of the crude protein, and the improved antioxidant activity increased the shelf life of lamb meat. Also, in Chios lambs, administering oregano EO (1 mL/kg) of concentrated feed resulted in strong antioxidant effects, retarding lipid oxidation (MDA formation) in meat during refrigeration and long-term frozen storage [75]. However, similarly to Muñoz-Cuautle et al. [74], in this experiment too, the EO administration did not result in improved final body weight, body weight gain, carcass yield, and tenderness of the *Longissimus thoracis* muscle. The supplementation of oregano EO at 300 mg/kg exerted an improved growth and slaughter performance in Sewa lambs, suggesting that this dose could have a fattening effect on lambs [63]. However, in this case, oregano EO was sprayed on a carrier, consisting of gelatine, sucrose, starch, and dextrin, diluted to an appropriate amount with carrier, mixed in a premix, and then added to the diet.

Ruminally cannulated lactating dairy cows supplemented with monensin, oregano oil, and carvacrol (50 mg/kg of DM) did not exhibit increased milk yield or improved consistency in milk composition; there were no effects on DMI and ruminal fermentation [76]. Also, the administration of increasing amount of oregano leaves (up to 750 g/d; 12 g/d EO; 9 g/d carvacrol) in dairy cows led to no changes in milk yield and milk components [77]. However, the dietary administration of 1200 mg of rosemary extract increased milk performance, considering the high productivity of Valle del Belice ewes, and also sustained body condition score [78]. Moreover, dairy ewes of the Chios cross-breed treated during summer by adding a specific cornus, a Mediterranean plant rich in anthocyanins, gallic acid, and ellagic acid with a high antioxidant activity, into the EO compounds of oregano and thyme, displayed higher milk production, lower milk urea concentration, and SCC [79]. In addition, the supplementation of cornus hydrodistillation extract, as well as oregano and thyme essential oils, improved the oxidative stability of milk, even though it did not affect the milk fatty acid profile. Addition of a specific mixture of EO compounds, especially at doses of 100 and 150 mg/ewe per day, had significant effects on milk production, urea concentration, and SCC in milk samples of Chios lactating sheep [48]. The supplementation was a mixture of natural and nature-identical EO compounds, including thymol, eugenol, vanillin, guaiacol, and limonene, bound to a specific organic–inorganic carrier containing benzoic acid, which ensured high stability of the effective components during storage and feed processing. However, the inclusion of Aloe vera extract at 20 and 40 g/kg DMI in the diet of lactating goats improved milk yield, enhanced antioxidant status, and increased rumen fermentation efficiency, with no negative effects on DMI and nutrient utilization [80]. The erythrocyte antioxidant enzyme activity and ferric-reducing total antioxidant power were higher after the use of Aloe vera extract, which resulted in an improvement in the antioxidant status of goats, thus increasing lactation performance; particularly, the best response was achieved with the supplementation of Aloe vera at 40 g/kg DMI. Goats supplemented with Aloe vera had increased milk production, fat, and protein content of milk by about 6% [80]. Very few studies investigated the effects of EO on milk components and milk fatty acid (FA) composition. Likewise, other studies reported none or only minor effects on milk FA in dairy cows fed with EO. However, Nudda et al. [81] suggested that the presence of higher feed rumen passage rate in small ruminants than large ruminants can limit the ability of rumen bacteria to complete the biohydrogenation process. Therefore, the administration of EO to small ruminants can contribute to an increase in the unsaturation of FA and conjugated linoleic acid (CLA), and this can be a commercially added value for small ruminant milk and cheese from a human diet and nutraceutical point of view. As a matter of fact, dietary orange peel EO supplementation in lactating Chios ewes' diet at levels up to 300 mg/kg concentrate, increased milk yield, fat yield, and ash yield, but decreased milk unsaturated (UFA) concentration and atherogenic index, whereas the orange EO supplementation at a higher level (450 mg/kg concentrate) had no effect. In the same study, the inclusion of 450 mg orange peel EO/kg concentrate improved blood plasma and milk antioxidant status [82]. In goats, the supplementation of 10 and 20% of distilled *Rosmarinus officinalis* spp. leaves succeeded in improving health features and the technological characteristics of goat milk [83]. In particular, rosemary supplementation at 20% reduced the milk clotting time, dry matter, and lactose milk content, C10 and C14 contents, and increased the percentage of C17, C18:2 and polyunsaturated FA; the 10% supplementation decreased the percentage of C14 and increased the C18:2 and PUFA content. The increase in C18:3, c9,t11-CLA, and monounsaturated FA concentrations in goat milk was also observed with the inclusion of a mixture of α-pinene, limonene, and β-caryophyllene in the diet [84]; the same increase in rumenic acid (c9,t11)-CLA and MUFA concentrations, together with C18, t10,c12-CLA, and PUFA, in goat milk was the result of the inclusion of garlic oil (0.57, 1.14, or 1.71 g/kg DM) in the diet of early lactating goats [85]. The inclusion of rosemary or lemongrass herbs (10 g daily) in the diet of lactating Damascus goats increased milk yield, energy-corrected milk yield, yield of milk components, and milk concentration of total solids, solids-not-fat, fats, and lactose, compared with the control treatment [86]. The augmented acetate production in the rumen of goats fed with rosemary and lemongrass could enhance the milk fat content, which mainly depend on dietary feed. In addition, the phenolic compounds contained in rosemary and lemongrass may have inhibited the bacterial activity of rumen bacteria involved in the biohydrogenation of PUFA in the diet and improved the efficiency of fatty acid absorption from the gut. As a result, milk from goats feed with rosemary and lemongrass had enriched PUFA, decreased saturated fatty acids (SFAs), and an increased ratio of UFA and SFA. In particular, an increase in CLA was observed which led to consider rosemary and lemongrass as useful supplements to increase the nutraceutical features of goat milk [86]. These results are confirmed also by the study of the administration of cumin (*Cuminum cyminum*) seed extract (at 1.27% and 2.53% of dry matter intake) to lactating crossbred goats (Alpine × Beetal); cumin extract decreased the growth of rumen *Prevotella brevis*, *Streptococcus bovis*, *Peptostreptococcus anaerobius*, and *Lachnospira multiparus*, which are involved in ruminal biohydrogenation [87]. As a result, even though the growth of *Butyrivibrio* sp. was not inhibited by cumin extract, the altered bacterial biohydrogenation activity led to an increase in rumen PUFA, thus increasing rumenic acid by 34.8%, vaccenic acid by 11.4%, and enhancing milk production by 13%, particularly when goats were supplemented with 1.27% of cumin extract [84]. While no effect on milk fat, protein, and lactose percentage was observed, MUFA, PUFA and the ratio PUFA to SFA content of milk significantly increased when additive was used at both levels. The cis-9 trans-11 CLA increased by 20% in goat milk receiving the cumin seed extract supplementation in the diet, as confirmed with a higher Delta-9-desaturase index in comparison to the control.

It seems that both the composition of fatty acids of milk and the dairy products derived from them can be positively modified with the administration of EO in diet. As seen, the administration of 20% rosemary EO in goat diet increased the protein content of P.O.D. Murcia al Vino cheese, without affecting cheese yield, microbiological count, and sensory parameters [83]. The addition of cornus hydrodistillation extract, as well as oregano and thyme essential oils, improved the oxidative stability of Feta cheese and yoghurt, with no effects on their fatty acid profile [79].

Based on the literature summarized, it appears that the precise mechanisms of action responsible for the observed improvements in ruminant health and milk quality is still inconclusive. Especially, knowledge of the specific biochemical and molecular mechanisms of action of EOs in in vitro studies on animal cells and ruminal microbial diversity is needed. To this end, it is crucial to develop more standardized methods to extract EO and find a safe EO dosage for administration in animal diet. Subsequently, new in vivo trials should be performed to test the proper effects and doses in relation to the in vitro experiments, as well as to test the effects based on the specific output required, such as the reduction in GHG production and products oxidation, and the improvements in immune responses, immune competence, and animals' performances.

5. Conclusions

The present review reports on the findings concerning the role of EOs in improving rumen fermentation efficiency, altering gut microbiota, and supporting small ruminant-derived products. On rumen microbiota, EOs can affect both the abundance and the type of microorganism, depending on the dose and type of administration, which in turn could alter rumen functioning and GHG production. Moreover, EO is found to increase the ruminal absorption of total volatile fatty acid concentration, with better feed conversion ratio. EOs can change the intestinal microbiota, and can thus improve the integrity of intestinal barrier, gut immune responses, and growth performance.

As evidenced by the present review, additional research is necessary to increase the knowledge on the role of EOs in sustaining small ruminants' health and welfare. Certainly, more standardized methods are direly required to produce and normalize the use of EOs in small ruminants' diet. The precise mechanisms of action responsible for the observed improvements in ruminant health and milk quality need to be further elucidated. The current review highlights an urgent need of in vitro and in vivo studies to define the effects of EOs as antimicrobial, antioxidant, and anti-inflammatory modulators in small ruminants.

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