Effect of essential oils on ruminal fermentation: A review

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ABSTRACT

Microbial digestion in the rumen is a complex phenomenon that efficiency can be improved by the use of food additives. The use of natural additives in ruminants can act on the balance of the ruminal population, and fermentation is directed towards the formation of end products that must be used more efficiently by the animal. Flora regulators, which are antibiotic in nature, have been widely used, but their use is now prohibited in livestock farming because of their harmful effect on public health. An alternative is to use natural product like essential oil whose antibacterial activity and effect on ruminal fermentation has been demonstrated. These are mixtures of lipophilic, liquid and volatile products present in higher plants used for their antiseptic and antibacterial properties over a broad bacterial spectrum. Antibacterial activity appears to result from a combination of several modes of action, involving different cellular targets and depends on several parameters such as the type of microorganisms tested, the composition of the EO and the method used. Use of essential oils (EOs) to modulate rumen microbial fermentation process to improve feed utilization efficiency and decreasing methane emission. This review explores the informations available in the literature regarding EO and their majority components and their mode of action, antimicrobial activity on rumen fermentation and methane reduction.

Keywords: *Essential oil, majority components, Rumen fermentation, Methane, Antibacterial.*

INTRODUCTION

Essential oils are known for their astringent and antibacterial properties. They are used as additives in ruminant feed, to modify the orientation of rumen fermentations and to improve animal nutrition. Ruminal fermentation is a process that converts ingested feed into energy sources for the animal and ruminal bacteria degrade nutrients to produce volatile fatty acids and synthesize microbial protein as an energy and protein supply for the ruminant. However, this fermentation process has energy (losses of methane) and protein (losses of ammonia) inefficiencies that may limit production performance and contribute to the release methane (Demeyer.,2000; Haque.,2018). Several Studies have been conducted on the ability of essential oils of aromatic plants and their majotity components to reduce enteric methane production (Dorman et al., 2000; Ultee *et al.,* 2002; Chaves., 2008; Laabouri et al., 2017; Hamdani et al., 2019). Most studies suggest that their effect is mainly related to their chemical structure and the concentration used. Due to this natural biocidal character, it appeared important to evaluate the effect of EO and aromatic compounds from EO on the activity of rumen flora. A number of studies have examined effects of EO, and their active components, on rumen microbial fermentation (Hart., 2008; Patra et al., 2013; Hamdani., 2019) EO and their components have the potential to improve energy utilization in ruminants. Antimicrobial activities of EO have been demonstrated against a wide variety of microorganisms, including Gram-positive and Gram-negative bacteria (Machboeuf et al., 2007; Calsamigliaet al., 2007). This effect is attributed to a number of terpenoid and phenolic compounds contained in their structure (Chao et al., 2000; Greathead., 2003; Tamminga., 2007), At a high doses, EO can inhibit the process of ruminal fermentation.(Machboeuf., 2008).

The objective of the present paper is to review current knowledge and assess the potential effects of essential oils and their active components on ruminal fermentation and microbial population on ruminant after citing a brief reminder of the definition of EO, their mode of action, antimicrobial potency and parameters involved in this activity.

Definition of essential oil

Essential oil (EO) is defined as: "a product obtained from a vegetable raw material, either by steam distillation or hydrodistillation (Speranza and corbo., 2010).

Essential oils represent a complex mixture of chemical molecules that can contain more than sixty different components, of which two or three are major components constituting 20 to 70% of the mixture compared to others that are most often found in trace form. For example, carvacrol and thymol are the major components of Origanum compactum oil, linalol for Coriandrum sativum oil, menthol and menthone for Mentha piperita oil. Generally these major components determine the biological properties of the essential oil (Bakkali et al.,2008; Dhifi ., 2016).

Most components of EO are included in two groups: terpenoids and phenylpropanoids, both of which are synthesized through two separate metabolic pathways (Calsamiglia., 2007; Bakkali et al., 2008). **Terpenoids** represent the most diversified group of secondary metabolites plant; more than 15,000 different compounds are described in the literature. They are derived from a five-carbon base structure (C5H8), commonly referred to as isoprene. Depending on the repetitive number of this unit, terpenoids are classified into: monoterpenoids (C10), sesquiterpenoids (C15) and diterpenoids (C20) (Calsamiglia., 2007 ; Benchaar et al., 2008). . In the composition of most essential oils, monoterpenoids and sesquiterpenoids form the major part (Benchaar et al 2011; Calo et al., 2015).

Phenylpropanoids are less frequent compared to terpenoids. Nevertheless, some plants possess these compounds in significant proportions (Speranza and corbo., 2010). Phenylpropanoids are mainly derived from phenylalanine (Sangwanet al., 2001).

Mechanisms of action of essential oils

The mode of action of essential oils on bacterial cells is not clearly determined (Kalemba and Kunicka, 2003; Burt, 2004; Tabasum and vidyasagar., 2013). Due to the diversity of molecules present in oils, antibacterial activity seems to result from a combination of several modes of action, involving different cellular targets.

The main mechanisms and sites of action of the different components of essential oils are:

Cell wall alteration (Zhang et al., 2016 and 2017)

Degradation of the cytoplasmic membrane (Ultee et al., 2002; Amstrong ., 2006; Speranza and corbo., 2010);

Alteration of proteins membrane, Cell content leakage (Cox et al., 2000; Lambert et al., 2001 ;Hyldgaard et al.,2012).

Coagulation of the cytoplasm (Burt.,2004; Hyldgaard et al.,2012).

The exhaustion of the force of proton movement (Ultee et al., 2001; Ultee et al., 2002). Due to their hydrophobic nature, EOs can be inserted into the lipid layers of the bacterial cell membrane and mitochondria, disrupting structures and making them more permeable (Knobloch et al., 1989;Lambert.,2001; Hyldgaard et al.,2012;). Ions and other cell components may then leak (Cox et al., 2000; Carson et al., 2002).

EO inhibits bacterial ATP synthetases and causes a decrease in intracellular ATP production (Gill and Holley, 2004 and 2006; Perricone.,2015).The EOs seem to alter the permeability of the plasma membrane and cause loss of intracellular material. In addition, after crossing the membrane, it can interact with intracellular sites. EO causes a decrease in bacterial size, alteration of the bacterial wall, aggregation of the cytoplasm, and cell rapprochement, (Rasooli et al., 2006).

Antimicrobial activity of EO

The evaluation of the bacteriostatic and bactericidal effect of EOs has been the subject of several publications. The EOs most studied in the literature for their antimicrobial properties belong to the Labiatae family: thyme, oregano, lavender, mint, rosemary, sage, etc. Some studies have reported that thyme EO, as well as thymol, its major compound, inhibits the growth of Salmonella typhimurium, Staphylococcus aureus, Escherichia coli and other bacterial species (Horvath., 2010 ; Bazargani.,2016). Carvacrol is the main compound of thyme EO and oregano. It has been shown to be effective in the treatment of upper respiratory tract infections and also on oral mucosal microbes (Didry et al., 1993). Mint and oregano EOs exhibit remarkable antibacterial activity on Gram-positive and Gram-negative strains (Mkaddem et al., 2009; Pesavento et al., 2015). Dorman and Deans (2000) also showed that oregano EO significantly inhibits the growth of 25 infectious microorganisms. Similarly, Skandamis et al. (2002) reported that oregano EO is an excellent antibacterial agent, effective on Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella pullorum, and other pathogenic bacteria. Indeed, the broad spectrum of in vitro antimicrobial activity of oregano EO is attributed to the large portion of phenolic derivatives it contains such as carvacrol and thymol (Manohar et al., 2001). In the same context, Rhayour et al (2003) reported that clove, oregano and most oregano and thymol EOs have a bactericidal effect on Escherichia coli, Bacillus subtilis and Mycobacterium fortuitum.

In other studies Zhang et al (2016) tested cinnamon EO exhibited effective antibacterial against *Escherichia coli* and *Staphylococcus aureus* Cinnamon EO results in irregular cell metabolic activity, changes membrane permeability and membrane integrity of bacteria cells indicating cell damage.

These antibacterial effects of EOs have also been tested in vivo in animal models to treat certain experimental bacterial infections caused by Helicobacter pylori in mice (Bergonzelli et al., 2003; Ohno et al., 2003).
The antifungal properties of EO have been examined in vitro and in vivo by several author (Manohar et al., 2001; Bennis et al., 2004a; 2004b;Freire et al.,2017; Serra et al., 2018). Manohar (2001) demonstrated than oregano EO and the carvacrol it contains inhibited the growth of Candida albicans. Other work has shown fungicidal activity of eugenol and thymol in vitro on Saccharomyces cereviciae (Bennis et al., 2004a; 2004b). Similarly, studies by Chami et al. (2004a, 2004b, 2005a, 2005b), showed the fungicidal activity of eugenol and carvacrol on Candida albicans in vitro and in vivo in two animal models of oral and vaginal candidiasis. In recent study Massa (2018) evaluated the effect of 12 essential oils (tea tree, bay leaf, anise, basil, bergamot, lavender, mint, oregano, grapefruit, rosemary, winter savory and ginger) on susceptible strains C. glabrata strains resistant to the three predominantly used azole antifungals (clotrimazole, fluconazole, itraconazole). Oregano and winter savory were the two most effective essential oils causing growth inhibition and cellular damage to C. glabrata strains.
The use of EOs for their antiparasitic control effect has been reported in several studies. Hatimi et al, (2001) showed the anti-leishmanian activity of Artemisia herba-alba EO on two Leishmania species (Leishmania tropica and Leishmania major). Similarly, Santoro et al (2007) showed that oregano and thyme EOs inhibit the growth of Trypanasoma cruzi. Work conducted by Remmal et al, (2011) has shown an in vitro anti-parasitic action of clove, tea tree, thyme and wormwood EOs on Eimeria oocysts. Achahbar (2011) also demonstrated the efficacy of clove, thyme and wormwood EOs and thymol and eugenol in the preventive and curative treatment of experimental coccidiosis in broilers. Dudko et al (2018) showed that the inclusion of the preparation containing the essential oil blend of O. vulgare and Citrus spp. in the diet of sheep resulted in decreases in both the intensity and prevalence of coccidian infection within the flock as well had influence increases in lamb growth

The parameters involved in the biological activity of EO
The antimicrobial activity of EOs depends on several parameters such as the type of microorganisms tested, the composition of the EO and the method used. The different microorganisms do not have a similar sensitivity to EO. The latest may be biocidal against some strains, biostatic against others or have no effect . This is why it is important to mention the complete name and the Gram of the microorganisms. Bacillus subtilis and Staphylococcus aureus (Gram+), Escherichia coli and Pseudomonas aeruginosa (Gram-), Candida albicans (Yeast) and Aspergillus niger (fungi) are among the most studied microorganisms. Fungi generally show higher sensitivity than bacteria (Cox et al., 2000). Satrani et al. (2007) showed that bacteria were more vulnerable than fungi to the EO of wild chamomile (Cladanthus mixtus). This result is explained by the fact that terpenols are abundant compared to terpenic hydrocarbons.

Regarding Gram staining of bacteria, Gram- bacteria appear more resistant than Gram+ to EOs due to the structure of their outer membrane (Cox et al., 2000). Thus, the outer membrane of Gram- is richer in lipo-polysaccharides and proteins than that of Gram+ bacteria, which makes it more hydrophilic and prevents hydrophobic terpenes from adhering to it. Nevertheless, some low molecular weight phenolic compounds such as thymol and carvacrol can adhere to these bacteria by binding to proteins and membrane lipopolysaccharides through their functional groups and thus reach the more vulnerable inner membrane (Dorman and Deans., 2000; Zayyad et al., 2013; Jamali et al., 2014).

The biological activity of an EO is related to its chemical composition, the nature of the functional groups of the majority compounds (alcohols, phenols, terpenic and ketone compounds), and their proportions. The activity of an EO is often due to the activity of its major compounds. These compounds, evaluated separately as purified compounds, confirm or refute the activity of an EO of similar composition. It is likely that minority compounds enter into synergy with majority compounds. In this way, the value of an EO depends on all its components and not only on its majority compounds (Lahlou., 2004).

The molecules known to be active are terpenoids, because saturated hydrocarbons and ionic acetates are inactive . The effect of terpenoids on isolated bacterial membranes suggests that their activity is a function of the lipophilic properties of their terpenic constituents, the nature of the functional groups, their solubility in the aqueous phase and the stereochemistry of the molecule (Dorman and Deans., 2000).

The most effective and broad-spectrum chemical compounds are phenols (thymol, carvacrol and eugenol), alcohols (α-terpineol, terpinen-4-ol, linalol), aldehydes and ketones. Antifungal activity decreases with the type of chemical functions (Hernandez., 2005):Phenols >alcohols> aldehydes> ketones> ethers> hydrocarbons.

-The methods used to determine the antimicrobial potency of EOs have a significant influence on the results obtained. The practical difficulties come from the insolubility of the constituents of EOs in water, their volatility and the problems of standardization of methods. The existing methods are divided into 3 main categories:

- The microdilution method

This is a liquid method, the EOs are dispersed in a detergent solution before being introduced into the culture broth (Morris et al., 1979). By varying EH concentrations, minimum inhibitory concentrations (MICs) can be determined and by transplantation, minimum bactericidal concentrations (BMCs) can be determined. However, the work done by Remmal et al. (1993a, b) showed that detergents (such as tween 80 and 20, and triton 100), as well as solvents such as ethanol, have a depreciating effect on the effectiveness of EO, while agar at 0.2% gives a good emulsion of EO without altering the specific antimicrobial activity of the EO tested.

The disc method

This method consists of placing a disc of paper impregnated with a given amount of EO in the centre of a uniformly seeded agar surface with the suspension of the microorganism to be studied. This method reports the effect of the volatile fraction as well as the effect of the compounds in contact. Antimicrobial activity results in an inhibition zone around the disc. The larger the diameter of this area, the more sensitive the strain is to EO. The smaller it is, the more resistant the strain is (Fauchère and Avril., 2002). The disadvantage of this method is that EOs do not diffuse well in the agar.

- The microatmosphere method

The difference between this method and the previous one lies mainly in the position of the EO impregnated disc. In this method, the impregnated disc is placed in the centre of the cover of the Petri dish, reversed during the experiment. The latter is therefore no longer in contact with the agar medium (Billerbeck et al., 2002).

Effect of EO on rumen flora

The rumen is a complex microbial ecosystem composed of several categories of microbial populations: bacteria, archaebacteria, protozoa, fungi and viruses.
Rumen bacteria represent half of the microbial biomass, their concentration varies from 10^{10} to 10^{11} cells/ml. Depending on the nature of the mainly fermented substrate, these microorganisms are classified into several groups of bacteria: cellulolytic, amylolytic, proteolytic, lipolytic...(Fonty et al.,1995). Methanogenic microorganisms are exclusive members of the Archaea domain, they represent about 4% of the ruminal microbiota and can be distinguished from other microorganisms by the production of methane (CH4) as the main fermentation product (Janssen.,2008; Cersocimo and Wright., 2015).
Depending on the nature of the available fermentable nutrients and the physico-chemical characteristics of the environment, the nature and relationship between the different rumen populations can change radically, which influences the quality and quantity of the fermentation end products. For example, a ration rich in fodder promotes the development of cellulolytic flora while another rich in concentrates benefits amylolytic flora, (Jarrige et al., 1995; Russell.,2002; Aschenbach et al., 2014).Much research has focused on evaluating the potential use of essential oils (Eos) to modulate rumen microbial fermentation process to improve feed utilization efficiency decreasing methane emission. Mcintosh et al (2003) described the effect of a mixture of EO on pure bacterial cultures: Streptococcus bovis appears to be the most resistant, while Prevotella ruminicola, Clostridium sticklandii and Peptostreptococcus anaerobius are very sensitive to this mixture . However, the use of high concentrations of HES can inhibit the entire ruminal fermentation, suggesting a shift from selective to a more general effect leading to the inhibition of most microorganisms (Hart et al.,2008). Just as thymol has a selective effect at a low concentration by inhibiting the species Selenomonas ruminantium, while in a higher concentration all tested species are inhibited (Evans and Martin., 2000; Gunal et al 2017)). This indicates that the intensity of antimicrobial activity of HES is strongly related to the concentration used (Hart et al., 2008).
In an in vitro study conducted on pure cultures, the growth of the species Methanobrevibacter smithii is not inhibited at 160 ppm by a mixture of EO but is inhibited at a higher concentration (Amlan and Patra.,2010). According to the literature, the chemical nature of EO influences their activity on rumen bacteria. Busquet et al (2006) tested in batch culture, the EO of anise (Pinpinella anisum, 86% anethole), cade (Juniperus oxycedrus, 35% alpha pinene), cinnamon bark (Cinnamonum cassia, 59% cinnamaldehyde), cloves (Syzygium aromaticum, 85% eugenol) dill (Anethum graveolens, 47% decarvone), oregano (Origanum vulgare,69% carvacrol) and tea tree (Melaleucaalternifolia, 42% terpinene-4-ol). The inhibitory effect of these EOs at a dose of 3000 ppm on

rumen bacteria was assessed by measuring the production of VFA in the environment. Based on the observed decrease in production, EOs rank as follows from most to least inhibitory: oregano > cloves > cinnamon bark > aniseed > tea tree > cade > dill. This gives, taking into account the chemical family to which the EO belongs: phenol > cinnamaldehyde > phenolmethyl ether > monoterpenol > ketone > terpene hydrocarbon.
Patra and Yu (2013) showed in vitro that oregano, garlic and mint EOs, used at a dose of 0.50g/L fermentation medium, affected bacterial communities. Various EO have been individually evaluated to mitigate methane and ammonia production by rumen microbiota. Interactions between EO can affect their potency but such interactions largely remain unexplored. Cobellis et al (2016) tested, EO from oregano, rosemary, Ceylon cinnamon, cinnamon leaves, cinnamon bark, dill seeds, and eucalyptus *in vitro*, both individually (at 1.125 ml/L culture) and in three-way EO combinations (at total EO 0.8 ml/L, equal ratio), for their effects on fermentation, methanogenesis, ammoniagenesis, and bacteria and archaea. All the EO and their combinations decreased production of total gas ($P < 0.001$), methane ($P < 0.001$), and ammonia (except eucalyptus EO; $P < 0.001$), but they (except the Ceylon cinnamon-dill seeds-eucalyptus EO combination) also decreased dry matter digestibility ($P < 0.001$). The EO individually decreased the abundances of *Prevotella* spp. ($P < 0.001$) but only oregano EO reduced the abundance of archaea ($P < 0.001$). The EO combinations significantly decreased the abundances of archaea ($P < 0.001$), protozoa ($P < 0.001$), and select groups or species of different rumen bacteria to different extents.

Effect of essential oils on ruminal metabolism of nitrogen-containing

The production of ammonia, amino acids long- and short-chain peptides is used as an indicator to assess the proteolytic activity of microorganisms in the rumen. Mainly, the determination of ammonia is the most studied parameter because ammonia represents both the final product of the degradation of nitrogenous material and an essential component for growth for several microbial species such as cellulolytic bacteria.

The nature of the Eos and the concentrations used strongly influence their effect on protein metabolism. In an in vitro study and at different concentrations (5, 50, 500 mg/l), eugenol decreases ammonia production with all three doses, the effect of limonene appears at 500 mg/l while vanillin has no impact with all three concentrations (benchaar et al, 2008).

Essential oils have a selective effect allowing them to react differently on microorganisms that metabolize proteins. Hyper-producing bacteria of ammonia are present in the rumen in very small numbers but they are responsible for 50% of the deamination function (hart et al., 2008), this group contains several species that have a very variable sensitivity to EOs. In a study conducted on pure bacterial cultures, a mixture of EOs is able to inhibit the growth of some species of ammonia-hyper-producing bacteria such as Clostridium sticklandii and Peptostreptococcus anaerobius, while other species, such as Clostridium aminophilum, are less sensitive (MachIntoch et al., 2003). Protozoa also have strong proteolytic and deamination activity and their inhibition also influences protein degradation in the rumen (castillejos et al 2005, Macheboeuf.,2006).

The effect of EOs also varies with the nature of the substrates used and their protein content (castillejos et al 2005). A 77% reduction in ammonia-producing bacteria is observed in sheep that have received a low-protein ration with a 100 mg/day mixture of EOs, this effect is absent with a higher protein ration (Benchaar., 2008). In another study and in the presence of a mixture of EOs, the microbial degradation of proteins is more intense with rapeseed meal than with soybean meal (Newbold et al.,2004). The result founded *in vivo* showed that the addition of a mixture of Eos (2 g/day) to the cattle ration for 28 days has no effect on ammonia production (Benchaar et al 2006). However, the addition of a mixture of cinnamaldehyde (0.6 g/day) and eugenol (0.3 g/day) for 21 days results in a decrease in ammonia production (Cardozo et al 2006).

Effect of essential oils on digestibility and production of volatile fatty acids

The use of high concentrations of EOs strongly inhibits the ruminal flora, which reduces digestibility and production of VFA s (Oh et al 2016). The desired effects are most often observed at low and medium doses (Benchaar et al., 2008; Oh et al 2016. Nevertheless, the nature of the essential oil used plays a crucial role in this process, a concentration of 500 mg/l of eugenol does not affect the digestibility and production of VFA s while thymol at the same dose significantly decreases both(Castillejos et al., 2006). As well as a mixture of EOs (1.5 mg/l) increases the production of VFAs without reducing digestibility, after 8 days of fermentation in continuous culture (Castillejos.,2005).The effect of EOs can also vary with the nature of the substrate used, a study on the effect of a mixture of EOs shows an increase in VFAs in cattle by the use of silage forages, however, a significant decrease is observed with silage corn (Benchaar et al 2008)., The small change in the

production of total VFAs can be beneficial if accompanied by a change in their profile, by an increase in propionate production and a decrease in acetate production, which means a decrease in methane production(Benchaar et al 2008; Cobellis et al .,2016; Poudel et al 2018).

Effet on essential oil on rumen fermentation

Microorganisms in the rumen degrade nutrients to produce volatile fatty acids and synthesize microbial protein as an energy and protein supply for the ruminant. However, this fermentation process has energy (losses of methane) and protein (losses of ammonia N) inefficiencies that may limit production performance and contribute to the release of pollutants to the environment. Several studies have been carried out on the ability of EOs to reduce methane production and all the work suggests that the effect of EOs is mainly related to their chemical nature and the concentration used. Borchers(1965) was the first to report the potential benefit of essential oils on rumen microbial fermentation. Borchers observed that the addition of thymol (active compound of thyme and oregano) to rumen fluid reduced ammonia concentrations, suggesting that thymol inhibited deamination and methanogenesis.

Essential oils extracted from cinnamon, eucalyptus and peppermint also have a strong inhibition of ruminal methanogenesis (Agarwal et al., 2009; Chaves et al.,2008. Tatsuoka et al.,2008; Kung et al 2008;Patra and Yu.,2013 ;Cobellis.,2016). EOs extracted from eucalyptus reduced in vitro methane production by 58% at a concentration of 1.66 ml/l, and by 90.3% at a higher dose (Machboeuf et al., 2008). Several studies are being carried out on pure compounds of Eos alone or in mixtures. Thymol, carvacrol, cinnamaldehyde, eugenol and anethole are the most studied molecules in this field. They all have a very significant effect on methane reduction, which can reach total inhibition at high concentrations (Calsamiglia et al., 2007; Chaves et al., 2008; Machboeuf et al.,2008). The potential benefits of eugenol on rumen microbial fermentation were further tested on cattle *in vitro* by Castillejos et al. (2006) Therefore, it appears that eugenol may improve VFA production and the VFA profile in the rumen of lactating animals. Busquet et al. (2006) confirmed that clove bud oil affected rumen fermentation, reducing total VFA , methane production and ammonia concentrations.

Cardozo et al. (2004), in a continuous culture experiment, were the first to suggest that cinnamon oil (0.22 mg/L of rumen fluid) modified the rumen fermentation, but the effects on VFA concentration and methane production were negligible. The effect of CIN on ruminal rumen fermentation have been inconsistent on this study. Compared to other secondary plant metabolites (thymol or carvacrol), cinnamaldehyde did not affect membrane stability and suggested that its mechanism of action was related to its interaction with proteins in the periplasm or deeper parts of the cell (Nikaido, 1994). Hart et al (2019) evaluated *in vivo* the addition of EO to dairy cows, and they noted that Feeding EO to dairy cows reduced CH_4 emissions whilst also increasing performance. Hamdani et al (2019) showed *in vitro* a significant effect of thymol on methane production. A significant decrease ($p < 0.05$) in gas production was observed as the concentration of thymol additive increases from 2.5 mg/ml to 20 mg/ml. Hamdani et al (2019) demonstrated *in vivo* that the addition of thymol-based additive induces a significant methane reduction by about 23% when the product was added to the daily ration of cow at the 50g /head /day, and by about 33% when the dose was doubled (100g/head /day). Thymol is a molecule that possesses high antimicrobial properties It can be used for handling rumen fermentation. This suggests that Compounds with phenolic structures, such as thymol, are more effective as antimicrobials in comparison with other non phenolic secondary plant metabolites because of the presence of a hydroxyl group in the phenolic structure.

Conclusion

This review attemps to shed light on the potential of essential oil and their active componend on rumen fermentation bacterial flora and methane emission. Most essential oils and active components tested inhibited rumen microbial fermentation at different concentrations depending on the chemical composition of the majority compounds and the potential adaptation of rumen flora.

References

Agarwal N., Shekhar C., Kumar R., Chaudhary L.C., Kamra D.N., 2009. Effect of peppermint (*Mentha piperita*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. *Animal Feed Science and Technology.* 148: 321–327.

Amlan K. and Patra J.S., 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. *Phytochemistry*. 71 : 1198–1222.

Armstrong, j.s. Mitochondrial membrane permeabilization: the sine qua non for cell death. BioEssays, Cambridge, v.28, p.253-260. 2006.

Aschenbach J. R., Penner G. B., Stumpff F., & Gäbel, G. 2011. Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *Journal of animal science*, *89*(4), 1092-1107.

Bakkali F., Averbeck S., Averbeck D., Idaomar M., 2008. Biological effects of essential oils. *Food Chemical Toxicology*. 46 : 446–475.

Bazargani, M. M., & Rohloff, J. 2016. Antibiofilm activity of essential oils and plant extracts against Staphylococcus aureus and Escherichia coli biofilms. *Food control*, *61*, 156-164.

Benchaar C., Calsamiglia S., Chaves A.V., Fraser G.R., Colombatto D., McAllister T.A. *et al.*, 2008. Plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology*. 145 : 209–228.

Benchaar C., Petit H.V., Berthiaume R., Whyte T.D., Chouinard P.Y., 2006. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production and milk composition in dairy cows. *Journal of Dairy Science*. 89 : 4352–4364.

Bennis S., Chami F., Chami N., Bouchikhi T. and Remmal A. 2004a. Surface alteration of saccharomyces cereviciae induced by thymol and eugenol. Lett. Appl. Microbiol. 38:454-8.

Bennis S., Chami F., Chami N., Rayour K., Tantaoui-Elaraki A. and Remmal A. 2004b. Eugenol induces damage of bacterial and fungal envelope. Moroccan J. Biol. 1: 33- 39.

Bergonzelli G.E., Donnicola D., Porta N. and Corthésy-Theulaz I.E. 2003. Essential Oils as Components of a Diet-Based Approach to Management of Helicobacter Infection. *Antimicrob. Agents Chemother*. 47: 3240–3246.

Billerbeck V.G., Roques C.G., Bessiere J.M. Fonvieille J.L., Dargent R., 2001. Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger* Canadian Journal of Microbiology 47: 9–17

Borchers, R. 1965. Proteolytic activity of rumen fluid in vitro. J. Anim.Sci. 24:1033–1038.

Brunechon J. (1987) Pharmacognosie, Ecole technique de documentation, Ed. Ravoilie.

Burt S, 2004. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol*.94:223–253.

Busquet M, Calsamiglia S, Ferret A, Kamel C, 2006. Plant extracts affect in vitro rumen microbial fermentation. *Journal of dairy science*, *89*(2), 761-771.

Calo J. R., Crandall P. G., O'Bryan C. A., and Ricke S. C. 2015. Essential oils as antimicrobials in food systems–a review. *Food Control* 54, 111–119. doi: 10.1016/j.foodcont.2014.12.040

Calsamiglia S., Busquet M., Cardozo P.W., Castillejos L., Ferret A., 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science*. 90 : 2580–2595.

Cardozo P.W., Calsamiglia S., Ferret A., Kamel C., 2006. Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. *Journal of Animal Science*. 84 : 2801–2808.

Carson C. F., B. J. Mee and T. V. Riley 2002. Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus ureus determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob. Agents Ch.46:1914-1920.

Castillejos L., Calsamiglia S., Ferret A., 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. *Journal of Dairy Science*. 89 : 2649–2658.

Castillejos L., Calsamiglia S., Ferret A., Losa R., 2005. Effects of a specific of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. *Animal Feed Science and Technology*. 119 : 29–41.

Cersosimo L. M., and Wright A. D. G. (2015). Rumen methanogens. In *Rumen Microbiology: From Evolution to Revolution* (pp. 143-150). Springer, New Delhi.

Chami F., Chami N., Bennis S., Bouchikhi T. and Remmal A. 2005a. Oregano and clove essential oils induce surface alteration of Saccharomyces cerevisiae, Phytotherapy *reasearch*. 19: 405-8.

Chami F., Chami N., Bennis S., Trouillas J. and Remmal A. 2004a. Evaluation of carvacrol and eugenol as prophylaxis and treatment of vaginal candidiasis in an immunosuppressed rat model. *J. Antimicrob. Chemother*. 54: 909-914.

Chami N., Bennis S., Chami F., Aboussekhra A. and Remmal A. 2005b. Study of anticandidal activity of carvacrol and eugenol in vitro and in vivo. Oral. *Microbiol. Immunol*. 20: 106-111.

Chami N., Chami F., Bennis S., Trouillas J. and Remmal A. 2004b. Antifungal treatment with carvacrol and eugenol of oral candidiasis in immunosuppressed rats. *Braz. J. Infect. Dis*. 8:217-26.

Chao S. C., Young D. G., & Oberg C. J. 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *Journal of essential oil research*, *12*(5), 639-649.

Chaves A.V., He M.L., Yang W.Z., Hristov A.N., McAllister T.A., Benchaar C., 2008. Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. *Canadian Journal of Animal Science*. 89 : 97–104.

Cobellis G., Trabalza-Marinucci M., Marcotullio M. C., & Yu, Z. 2016. Evaluation of different essential oils in modulating methane and ammonia production, rumen fermentation, and rumen bacteria in vitro. *Animal Feed Science and Technology*, *215*, 25-36.

Cox S.D., Mann C.M., Markham J.L., Bell H.C., Gustafson J.E., Warmington J.R. and Wyllie S.G. 2000. The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil*). J. Appl. Microbiol.* 88: 170-175.

Demeyer D, and fievez V, 2000. Ruminants et environnement: la méthanogenèse. *Ann. Zootech.* 49 : 95-112.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., & Mnif, W. 2016. Essential oils' chemical characterization and investigation of some biological activities: a critical review. *Medicines*, *3*(4), 25.

Didry N., Dubreuil L., and Pinkas M. 1993. Antimicrobial activity of thymol, carvacrol and cinnamidaldehyde alone or in combination. *Pharmazie*, 48; 301-304.

Dorman H.J.D. and Deans S.G. 2000 .Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol*. 88: 308-16.

Dudko P., Junkuszew A., Bojar W., Milerski M., Szczepaniak K., Le Scouarnec, J., Grzybek, M. 2018. Effect of dietary supplementation with preparation comprising the blend of essential oil from Origanum vulgare (lamiaceae) and Citrus spp.(citraceae) on coccidia invasion and lamb growth. *Italian Journal of Animal Science*, *17*(1), 57-65.

Evans J D, Martin S A, 2000. Effects of thymol on ruminal microorganisms. Current Microbiology. 41 : 336–340.

Fauchère J.L. et Avril J.L. 2002. "Bactériologie générale et médicale" Ellipses Editions Paris, 365p.

Fonty G., Jouany J.P., Forano E. *et al.*, 1995. L'écosystème microbien du réticulo-rumen. *In*: Jarrige R., Ruckebusch Y., Demarquilly C. *et al*. Nutrition des ruminants domestiques, ingestion et digestion. INRA (Eds.). Paris, France. 299–348.

Freire J. C. P., Júnior J. K. D. O., Silva D. D. F., Sousa J. P. D., Guerra F. Q. S., Oliveira Lima, E. (2017). Antifungal Activity of Essential Oils against Candida albicans Strains Isolated from Users of Dental Prostheses. *Evidence-Based Complementary and Alternative Medicine*, *2017*.

Gill, A. O., & Holley, R. A. (2004). Mechanisms of bactericidal action of cinnamaldehyde against Listeria monocytogenes and of eugenol against L. monocytogenes and Lactobacillus sakei. *Appl. Environ. Microbiol.*, *70*(10), 5750-5755.

Gill A. O., & Holley R. A. 2006. Disruption of Escherichia coli, Listeria monocytogenes and Lactobacillus sakei cellular membranes by plant oil aromatics. *International journal of food microbiology*, *108*(1), 1-9.

Greathead H. 2003. Plants and plant extracts for improving animal productivity. *Proceedings of the Nutrition Society*, *62*(2), 279-290.

Günal M., Pinski B., AbuGhazaleh, A. A. 2017. Evaluating the effects of essential oils on methane production and fermentation under in vitro conditions. *Italian Journal of Animal Science*, *16*(3), 500-506.

Gustafson J. E., Y. C. Liew S. Chew, J. L. Markham H. C. Bell, S. G. Wyllie and J. R. Warmington 1998. Effects of tea tree oil on Escherichia coli Lett. Appl. Microbiol. 26:194-198.

Haque M. N. 2018. Dietary manipulation: a sustainable way to mitigate methane emissions from ruminants. *Journal of animal science and technology*, *60*(1), 15.

Hart K. J., Jones H. G., Waddams K. E., Worgan H. J., Zweifel B., & Newbold C. J. 2019. An Essential Oil Blend Decreases Methane Emissions and Increases Milk Yield in Dairy Cows. *Open Journal of Animal Sciences*, *9*(03), 259.

Hart K.J., Yánez-Ruiz D.R., Duval S.M., McEwan N.R., Newbold C.J., 2008. Plant extracts to manipulate rumen fermentation. *Animal Feed Science and Technology*. 147 : 8–35.
Hatimi S., Boudouma M., Bichichi M., Chaib N. and Idrissi N.G. 2001. Evaluation *in vitro* antileishmanial activity of *Artemisia herba-alba* Asso. *Therapeutic, Bull. Soc. Pathol.* Exot. 94: 29-31.

Helander, I. M., H. Alakomi, K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E. J. Smid, L. G. M. Gorris, and A. Wright. 1998. Characteritzation of the action of selected essential oil components on gram-negative bacteria. *J. Agric. Food Chem*. 46:3590–3595.
Horváth G., Jámbor N., Végh A., Böszörményi A., Lemberkovics É., Héthelyi É., Kocsis B. 2010. Antimicrobial activity of essential oils: the possibilities of TLC–bioautography. *Flavour and fragrance journal*, *25*(3), 178-182.

Houda Hamdani, Najat Chami, Mounia Oukhouia, Imane Jabeur, Chaimae Sennouni and Adnane Remmal 2019. Effect of a thymol-based additive on rumen fermentation, on methane emissions in eructed gas and on milk production in Holstein cows; *Livestock Research for Rural Development*. Volume 31, Article #107.

Hyldgaard M., Mygind T., & Meyer R. L. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in microbiology*, *3*, 12.

Jamali C. A., Kasrati A., Bekkouche K., Hassani L., Wohlmuth H., Leach D., & Abbad A. 2013. Phenological changes to the chemical composition and biological activity of the essential oil from Moroccan endemic thyme (Thymus maroccanus Ball). *Industrial Crops and Products*, *49*, 366-372.

Janssen A.M., Sheffer J.J.C. and Svendsen B. 1987. Antimicrobial activity of essential oils: A 1976-1985 literature review. Aspects on the test methods. *Planta Med.* 53: 395-508.

Janssen P.H., Kirs M., 2008. Structure of the archaeal community of the rumen. *Applied and Environmental Microbiology*. 74 : 3619–3625.
Jarrige R., Ruckebusch Y., Demarquilly C., Farce M.H., Journet M., 1995. Nutrition des ruminants domestiques, ingestion et digestion. INRA (Eds.).Paris. France. 270–362.
Juven B.J., Kanner J., Schved F. and Weisslowicz H. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *J. Appl. Bacteriol.* 76:626–631.

Knobloch K., Pauli A., Iberl B., Weigand H. and Weis N. 1989. Antibacterial and antifungal properties of essential oil components. *J. Essen. oil res.* 1: 119–128.

Kumar R., Kamra D.N., Agrawal N., Chaudhary L.C., 2009. Effect of *eucalyptus* (*Eucalyptus globulus*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. *Animal Nutrition and Feed Technology*. 9 : 237–243.
Kung Jr L, Williams P, Schmidt R J, Hu W, 2008. A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. *Journal of Dairy science*, *91*(12), 4793-4800.

Laabouri, F., Guerouali, A., Alali, S., Remmal, A., Ajbilou, M. (2017). Effet d'un additif alimentaire naturel riche en huile essentielle de thym sur les émissions de méthane chez les vaches laitières. *Revue Marocaine des Sciences Agronomiques et Vétérinaires*, *5*(3).

Lambert R.J.W., Skandamis P.N., Coote P.J. and Nychas G.J.E. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol*. 91 : 453-462.

Macheboeuf D., Morgavi D.P., Papon Y., Mousset J.L., Arturo-Schaan M., 2008. Dose-response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. *Animal Feed Science and Technology*. 145 : 335–350.
Macheboeuf D., Morgavi D.P., Papon Y., Mousset J.L., Arturo-Schaan M., 2008. Dose response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. *Anim. Feed Sci. Technol.,* 145, 335-350.
Macheboeuf D., Papon Y., Artuso-Schaan M., Mousset J.L., Cherel R., 2006. Utilisation d'extraits végétaux (huiles essentielles et extrait de polyphénols) pour diminuer la dégradation ruminale des protéines- Étude *in vitro*. *Rencontre Recherche Ruminants*. 13 : 69–72.
Manohar V., Ingram C., Gray J., Talpur N.A., Echard B.W., Bagchi D. et *al.* 2001. Antifungal activities of origanum oil against *Candida albicans*. *Mol. Cell. Biochem.* 228: 111–117.

Massa N., Cantamessa S., Novello G., Ranzato E., Martinotti S., Pavan M., Bona E. 2018. Antifungal activity of essential oils against azole-resistant and azole-susceptible vaginal Candida glabrata strains. *Canadian journal of microbiology*, *64*(10), 647-663.

McIntosh F.M., Williams P., Losa R., Wallace R.J., Beever D.A., Newbold C.J., 2003. Effects of essential oils on ruminal microorganisms and their protein metabolism. *Applied and Environmental Microbiology*. 69 : 5011–5014 *Microbiology*. 41 : 336–340.

Mkaddem M., Bouajila J., Ennajar M., Lebrihi A., Mathieu F., & Romdhane M. 2009. Chemical composition and antimicrobial and antioxidant activities of Mentha (longifolia L. and viridis) essential oils. *Journal of food science*, *74*(7), M358-M363.

Morris J.A., Khettry A. and Seitz E.W. 1979. Antimicrobial activity of aromachemicals and essentials oils. *J. Amer. Oil. Chem. Soc.* 56: 595-603.

Newbold C.J., McIntosh F.M., Williams P., Losa R., Wallace R.J., 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. *Animal Feed Science and Technology.* 114 : 105–112.

Nikaido, H. 1994. Prevention of drug access to bacterial targets: Permeability barriers and active efflux. *Science* 264:382–388.

Oh J., and Hristov A. N. 2016. Effects of plant-derived bio-active compounds on rumen fermentation, nutrient utilization, immune response, and productivity of ruminant animals. In *Medicinal and Aromatic Crops: Production, Phytochemistry, and Utilization* (pp. 167-186). American Chemical Society.

Panizzi L., Flamini G., Cioni P.L., Moreli I., 1993.Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. *J. Ethnopharmacol*. 39, 167–170. doi: 10.1016/j.scitotenv.2016.11.072.

Patra AK, and Yu Z, 2013. Essentials oils modify rumen bacterial compositions *in vitro* as revealed by microarrayanalysis. J. Anim. Sci., 91 (E-Suppl. 2/J), J. DairySci., 96 (E-Suppl. 1), pp. 713.

Perricone M., Arace E., Corbo M. R., Sinigaglia M., Bevilacqua, A. 2015. Bioactivity of essential oils: a review on their interaction with food components. *Frontiers in microbiology*, *6*, 76.

Pesavento G., Calonico C., Bilia A. R., Barnabei M., Calesini F., Addona R., Nostro, A. L. 2015. Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against Staphylococcus aureus and Listeria monocytogenes in beef meatballs. *Food Control*, *54*, 188-199.

Poudel P., Froehlich K., Casper D. P., & St-Pierre B. 2018. 438 Feeding an Essential Oils Blend to Neonatal Holstein Dairy Calves Increased Rumen Propionate Concentration and Resulted in Higher Representation of a Previously Uncharacterized Strain of Prevotella Ruminicola. *Journal of Animal Science*, *96*(suppl_2), 235-236.

Rasooli I., Rezaei M. B., Allameh, A. 2006. Growth inhibition and morphological alterations of Aspergillus niger by essential oils from Thymus eriocalyx and Thymus x-porlock. *Food Control*, *17*(5), 359-364.

Reichling, J., Schnitzler, P., Suschke, U., & Saller, R. 2009. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties–anoverview. *Complementary Medicine Research*, *16*(2), 79-90.

Remmal A., Achahbar S., Bouddine L., Chami N. and Chami F. 2011. *In vitro* destruction of *Eimeria* oocysts by essential oils. Vet. Parasitol. 182: 121-126.

Remmal A., Bouchikhi T., Tantaoui-Elaraki A. and Ettayebi M. 1993b. Inhibition of antibacterial activity of essential oils by tween 80 and ethanol in liquid medium. *J. Pharm. Belg.* 48: 352-356.

Remmal A., Tantaoui-Elaraki A., Bouchikhi T. and Ettayebi, M. 1993a .Improved method for the determination of antimicrobial activity of essential oils in agar Medium. *J. Essent. Oil. Res.* 5: 1179-184.

Rhayour K., Bouchikhi T., Tantaoui-Elaraki A., Sendide K. and Remmal A. 2003. The mechanism of bactericidal action of oregano and clove essential oils and of their phenolic major components on *Escherichia coli* and *Bacillus subtilis*. *J. Essent. Oil. Res.* 15: 356-362.

Romero-Pérez A., Alemu A., Araujo R., Beauchemin K. 2018. Effect of slow release nitrate and essential oil on animal performance and methane emissions from feedlot cattle fed high-grain finishing diets. *Journal of Animal Science*, *96*(suppl_3), 409-409.

Russell J. B. 2002. *Rumen microbiology and its role in ruminant nutrition*. Cornell University.

Sangwan N.S., Farooqi A.H.A., Shabih F., Sangwan R.S., 2001. Regulation of essential oil production in plants. *Plant Growth Regulation*. 34 : 3–21.
Santoro G.F., das Gracas M., Guimaraes L.G., Salgado A.P., Menna-Barreto R.F. and Soares M.J. 2007. Effect of oregano (*Origanum vulgare* L.) and thyme (*Th ymus vulgaris* L.) essential oils on *Trypanosoma cruzi* (Protozoa: Kinetoplastida) growth and ultrastructure. *Parasitol. Res.* 100: 783-790.

Serra E., Hidalgo-Bastida L., Verran J., Williams D., Malic S. 2018. Antifungal activity of commercial essential oils and biocides against Candida albicans. *Pathogens*, *7*(1),

Sivropoulou A., Papanikolaou, E., Nikolaou C., Kokkini S., Lanaras T., Arsenakis M. 1996. Antimicrobial and cytotoxic activities of Origanum essential oils. *Journal of agricultural and Food Chemistry*, *44*(5), 1202-1205.

Skandamis P., *Tsigarida E. and Nychas G.J.E.* 2002. *The effect of oregano essential oil on survival/death of Salmonella Typhimurium in meat stored at 5°C under aerobic, VP/MAP conditions. Food Microbiol.* 19:97-*103.*

Speranza B., Corbo M. R. 2010. Essential oils for preserving perishable foods: possibilities and limitations. *Application of alternative food-preservation technologies to enhance food safety and stability*, *23*, 35-37.

Tabassum N., Vidyasagar G. M. 2013. Antifungal investigations on plant essential oils. A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(2), 19-28.

Tamminga S., Bannink A., Dijkstra J., Zom R. L. G. 2007. *Feeding strategies to reduce methane loss in cattle* (No. 34). Animal Sciences Group.

Tatsuoka N., Koji H., Mikuni K., Kozo H., Zashimoto H., Itabashi H., 2008. Effects of the essential oil cyclodextrin complexes on ruminal methane production *in vitro*. *Animal Science Journal*. 79 : 68–75.
Ultee A., Bennik M .H.J., Moezelaar R., 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. Applied and EnvironmentalMicrobiology. 68 : 1561–1568.uptake by the ruminal bacterium *Selenomonas ruminantium*. *Appl. Environ. Microbiol*., 56, 3515-3518.

Ultee A., Kets E. P. W., Smid E. J. 1999. Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. *Appl. Environ. Microbiol.*, *65*(10), 4606-4610.

Xie Y., Wang Z., Huang Q., Zhang D. 2017. Antifungal activity of several essential oils and major components against wood-rot fungi. *Industrial Crops and Products*, *108*, 278-285.
Zayyad N., Farah A., Bahhou J. 2014. Analyse chimique et activité antibactérienne des huiles essentielles des trois espèces de Thymus: Thymus zygis, T. algeriensis et T. bleicherianus. Chemical analysis and antibacterial activity of essential oilsfrom three species of Thymus: Thymus zygis, T. algeriensis, and T. bleicherianus. *Bulletin de la Société Royale des Sciences de Liège*, *83*, 118-132.
Zhang Y., Liu X., Wang Y., Jiang P., Quek S. 2016. Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. *Food Control*, *59*, 282-289.