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# Effects of Essential Oils on Rumen Fermentation, Microbial Ecology and Ruminant Production

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#### ABSTRACT

Essential Oils (EO) are volatile aromatic compounds extracted from whole plants and are secondary metabolites usually made up of terpenoids and phenylpropanoids. Plant EO has antimicrobial properties, which can be effective against undesirable rumen microbes. Therefore, recently it has been great interests among nutritionists and rumen microbiologists to exploit EO as natural feed additives to improve rumen fermentation such as volatile fatty acids production, inhibition of methanogenesis, improvement in protein metabohsm and efficiency of feed utilization and increasing conjugated linoleic acids in ruminant derived foods. Different types of EO from a wide range of herbs and spices have been identified to have potential for rumen manipulations and enhancing animal productivity as alternatives to chemical feed additives. However, their effectiveness in ruminant production has not been proved to be consistent and conclusive. There are varying reports of EO on rumen fermentation, rumen microbiota and ruminant performance depending upon the dose, chemical structures of EO, feed composition and animal physiology, which have not always been adequately described in the literature. The comprehensive research based on individual components of EO, physiological status of animals, nutrient composition of diets and their effects on rumen microbial ecosystem and metabolism of EO will be required to obtain consistent beneficial effects.

Key words: Essential oils, rumen fermentation, rumen micro-organisms, ruminant performance

#### INTRODUCTION

For the past few decades, a number of chemical feed additives such as antibiotics, ionophores, methane inhibitors and defaunating agents have been tried in ruminant nutrition to modulate rumen fermentation, to enhance growth and milk yield and to improve feed intake and efficiency. But, most of these supplements are not used routinely because of toxicity problems to the host animals and microbial adaptation to these additives. Most importantly, a great awareness from public health aspects such as residues of these chemicals in milk and meat and bacterial resistance to antibiotics as a result of increased use in the food chains prohibits their use as feed additives (Barton, 2000). These supplements have been criticized by the consumers' organizations on the ground of product safety and quality. The consumers' demands have stimulated to search for natural alternatives to chemical feed additives. As plants are part of herbivore diets, plants those that contain bioactive compounds such as Essential Oils (EO), saponins and tannins with antimicrobial properties could be explored in animal nutrition to improve the feed utilization and health (Cowan, 1999). Therefore, recent research has been greatly focused to exploit plant bioactives as natural feed additives to improve rumen fermentation such as enhancing protein

metabolism, decreasing methane production (Wallace et al., 2002; Kamra et al., 2008; Patra and Saxena, 2009a, 2010) and reducing nutritional stress such as bloat and improving animal health and productivity (Patra, 2007). A number of recent reviews discussed the effects of EO on rumen fermentation (Calsamiglia et al., 2007; Hart et al., 2008; Benchaar et al., 2008) and rumen micro-organisms (Hart et al., 2008; Patra and Saxena, 2009b; Benchaar et al., 2008) and ruminant performance (Benchaar et al., 2008). Therefore, this review summarizes the effects of EO on rumen fermentation, microbial populations and ruminant performance such as growth, milk production and the efficiency of feed utilization and recent developments in the areas of rumen fermentation such as methane inhibition and increasing the content of conjugated linoleic acids, the health promoting fatty acids, in milk and meat.

# CHEMISTRY OF ESSENTIAL OILS

Essential oils are steam-volatile or organic-solvent extracts of plants. They are commonly derived from herbs and spices. They are also present to some degree in many plants, which serve as a protective role against bacterial, fungal or insect attack. Uses of EO as food preservatives and folk medicines are known for many centuries because of their antimicrobial effects. The most commonly occurring EO are included in two chemical groups: terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids, which are synthesized through the mevalonate and shikimic acid metabolic pathways, respectively (Gershenzon and Croteau, 1991; Calsamiglia et al., 2007). Among these two classes, terpenoids are the more diversified group of plant bioactives abundantly found in many herbs and spices (Gershenzon and Croteau, 1991). These compounds derive from a basic structure of C5 isoprene units and are classified depending on the number of these units in its skeleton. Within terpenoids, the most important components of EO of the majority of plants belong to the monoterpenoids and sesquiterpenoids (Gershenzon and Croteau, 1991; Calsamiglia et al., 2007). Phenylpropanoids have a side chain of 3 carbons bound to an aromatic ring of C6 (Calsamiglia et al., 2007). Phenylpropanoids are less abundant compounds of EO compared with terpenoid family, but some plants contain in significant proportions.

Essential oils are present in different parts of the plants such as flowers, leaves, bark, fruit pulps, roots, seeds and stems. The concentrations of EO vary due to stage of growth, plant health and environmental factors such as light, temperature and moisture stress (Hart *et al.*, 2008).

# ESSENTIAL OILS ON RUMEN MICROBIAL POPULATIONS

Essential oils were examined many years ago in ruminal bacteria from the point of view of the oils contributing to poor palatability in some plant species (Oh *et al.*, 1968). Because many EO compounds have strong antimicrobial properties, research to exploit EO as feed additives in animal nutrition has been accelerated recently due to the ban of some antibiotic growth promoters as feed additives in many developed countries.

Rumen bacteria: Essential oils might inhibit the Hyper-Ammonia Producing (HAP) bacteria in the rumen, which results in decreased amino acids deamination (Wallace, 2004; Patra and Saxena, 2009b). McInotch *et al.* (2003) observed that a mixture of EO inhibited the growth of some HAP bacteria (i.e., Clostridium sticklandii and Peptostreptococcus anaerobius), but other HAP bacteria (e.g., Clostridium aminophilus) were less sensitive. Inhibitory effects of EO on HAP bacteria may be diet dependent. For instance, Wallace (2004) reported that the number of HAP bacteria was reduced by 77% in sheep receiving a low protein diet supplemented with EO at 100 mg day<sup>-1</sup>, but

that EO had no effect on HAP bacteria when sheep were fed a high-protein diet. Total viable count of bacteria may not be unaffected by EO in that study. Individual EO had different effects on mixed ruminal bacteria. Monoterpene hydrocarbons were less toxic and sometimes stimulatory to microbial activity compared with the corresponding oxygenated compounds, the monoterpene alcohols and aldehyde (Oh et al., 1967, 1968). The HAP bacteria have a high capability to generate ammonia from amino acids (Wallace et al., 2002). At low doses, EO could selectively inhibit the HAP bacteria, but all micro-organisms are affected at higher concentrations. Evans and Martin (2000) reported that thymol selectively inhibited the growth of Selenomonas ruminantium effect at 90 mg L<sup>-1</sup>, but not S. bovis whilst at 400 mg L<sup>-1</sup> all rumen organisms tended to be inhibited. The EO might suppress the colonization and/or digestion of readily degradable substrates by amylolytic and proteolytic bacteria without affecting fibre digestion (Wallace et al., 2002). However, it had been noted that activities of carboxymethyl-cellulase and xylanase were reduced by extracts of clove and fennel (Patra et al., 2010) perhaps due to higher concentrations of EO present in the extracts.

Rumen protozoa: There are mixed reports on the effects on rumen protozoa. McInotch et al. (2003) observed that the bacteriolytic activity of rumen ciliate protozoa was unaffected in dairy cows supplemented with 1 g day<sup>-1</sup> of mixed EO. Similarly, Newbold et al. (2004) and Benchaar et al. (2007a) reported that ruminal protozoa counts were not affected when sheep and dairy cows were fed 110 and 750 mg day<sup>-1</sup> of a mixture of EO, respectively. Supplementation of dairy cows diets with 0.5 g of cinnamaldehyde per liter of rumen fluid had also no effect on the number of ciliate protozoa (Fraser et al., 2007). The extract of fennel had not effect on protozoa (Patra et al., 2010). In contrast Ando et al. (2003) reported that feeding 200 g day<sup>-1</sup> (i.e., 30 g kg<sup>-1</sup> of total dietary Dry Matter (DM) intake) of peppermint (Mentha piperita L.) to Holstein steers decreased the total number of protozoa and the numbers of Entodinum, Isotrica and Diplodium, which is attributed to the presence of EO. It had also been observed that clove extract containing EO decreased total numbers of protozoa, small entodiniomomphs and holotrichs, but did not affect large entodiniomorphs (Patra et al., 2010). However, Cardozo et al. (2006) observed that addition of a mixture of cinnamaldehyde (180 mg day<sup>-1</sup>) and eugnol (90 mg day<sup>-1</sup>) to the diets of beef heifers increased numbers of holotrichs and had no effect on entodiniomorphs, but there was no effect on numbers of these protozoal species when the mixture contained higher concentrations of cinnamaldehyde (600 mg day<sup>-1</sup>) and eugenol (300 mg day<sup>-1</sup>). Recently, Yang et al. (2010b) also observed that cinnamaldehyde supplemented with 0.4 to 1.6 g day<sup>-1</sup> in steers did not affect total protozoal as well as Isotricha, Dasytricha and Entodinium sp. In contrast, feeding 2 g day<sup>-1</sup> of anise extract containing 100 g kg<sup>-1</sup> of anethol to beef heifers decreased the counts of holotrichs and entodiniomorphs (Cardozo et al., 2006). Overall, EO and their components have no marked effects on numbers and/or activity of ruminal ciliate protozoa.

# ESSENTIAL OILS ON DIGESTIBILITY AND RUMEN FERMENTATION

Feed digestion: The main effects of EO in the rumen have been suggested to be due to reduction of protein and starch degradation and an inhibition of amino acid degradation due to selective action on certain rumen micro-organisms, specifically some bacteria (Hart et al., 2008). One mode of action suggested for EO is an effect on the pattern of bacterial colonisation of particular starch rich substrates as they enter the rumen. A second possible mode of action is the inhibition of HAP bacteria involved in amino acid deamination.

The digestibility of feeds was not affected in several studies (Meyer et al., 2009; Malecky et al., 2009; Santos et al., 2010). Yang et al. (2007) reported that ruminal digestibilities of DM were higher (13%) for juniper berry EO (2 g d<sup>-1</sup>) than for the control diet consisting of 40% forage and 60% barley-based concentrate in Holstein cows. However, total tract digestibilities of DM, organic matter, fiber and starch were not affected by experimental treatments. They suggested that increased ruminal digestibility was due to increased ruminal digestion of dietary protein by 11% as compared with the control. Malecky et al. (2009) also reported that a monoterpene blend (consisting of 45.2% linalool, 36.7% p-cymene, 16.0%  $\alpha$ -pinene and 2.2%  $\beta$ -pinene) did not affect digestibilities of different nutrients in dairy goats. Feeding of 500 mg ropadiar (containing volatile oils of marjoram) to sheep showed higher concentration of protein in the rumen fluid without affecting the nutrient digestibility (Kozelov et al., 2001). The higher concentrations of EO decrease the DM as well as fiber digestibility in the rumen (Beauchemin and McGinn, 2006; Yang et al., 2010b).

Volatile fatty acids: The total Volatile Fatty Acids (VFA) concentrations in the rumen were generally little affected (Chaves et al., 2008a; Malecky et al., 2009; Patra et al., 2010) or decreased (Macheboeuf et al., 2008; Kumar et al., 2009), especially at higher concentrations of EO. There are some studies showing increased concentrations of total VFA in the rumen due to supplementation of cinnamaldehyde at 0.2 g kg<sup>-1</sup> DM intake (Chaves et al., 2008b) and EO extract from oregano at 0.25 g kg<sup>-1</sup> DM intake (Wang et al., 2009). Castillejos et al. (2005) observed that a blend of EO added at 1.5 mg L<sup>-1</sup> increased total VFA without affecting nitrogen metabolism in dual flow continuous culture fermenters. Responses of EO on total VFA concentrations may depend upon the types of substrate fed to the ruminants. The total VFA concentrations were not affected in lactating cows fed on the alfalfa silage based diet, but were decreased fed on the corn-silage based diet with the addition of  $0.75 \text{ g day}^{-1}$  of an EO mixture (Benchaar et al., 2007a). The acetate to propionate ratios were increased (Benchaar et al., 2007b; Macheboeuf et al., 2008; Agarwal et al., 2009) or some times were not changed (Wang et al., 2009; Kumar et al., 2009). The inhibition of methane production in the rumen by specifically targeting the methanogens is usually associated with a decrease in acetate to propionate ratio. However, from a recent meta-analysis, it has been noted that the acetate to propionate ratio increased with the inhibition of methane by EO (unpublished data), which is not nutritionally favourable for energy utilization. The effects of EO may depend upon the pH of the ruminal fluid. Cardozo et al. (2005) found that some pure EO had a more pronounced impact on rumen VFA profiles at low rumen pH and proposed that the status of the EO molecules (i.e., dissociated or undissociated) is dependent on rumen pH. Similarly, Spanghero et al. (2008) also observed that a blend of EO shifted the end products of fermentation, in particular a reduction in the acetate proportion and the acetate to propionate ratio, but only at lower pH in the EO fluid.

Ammonia: As EO inhibit HAP bacteria in the rumen, the concentrations of ammonia and deaminase activities sometimes decreases. The HAP bacteria comprise only around 1% of the rumen bacterial populations, but they possess a very high deamination activity (Russell *et al.*, 1988; Wallace, 2004). This could decrease the rate of ammonia production in the rumen, which may be beneficial nutritionally by increasing the efficiency of protein utilization in the rumen (Wallace *et al.*, 2002). Newbold *et al.* (2004) reported a 25% reduction in bacterial deaminative activities *in vitro*. Ammonia concentrations were decreased *in vitro* with oregano oil at 30 and

300 mg  $L^{-1}$ , with cinnamon oil at 0.3-300 mg  $L^{-1}$  (pH 7.0; Cardozo *et al.*, 2005) and with cinnamaldehyde at 3000 mg  $L^{-1}$  (Busquet *et al.*, 2006). But these effects were not observed in some other studies *in vitro* with anethol up to 3000 mg  $L^{-1}$ , carvacrol and carvone up to 300 mg  $L^{-1}$  (Busquet *et al.*, 2006) and *in vivo* (Castillejos *et al.*, 2005; Benchaar *et al.*, 2007a).

Some EO compounds decreased ammonia concentrations at low doses compared with other EO compounds. Guaiacol lowered ammonia concentrations as  $5 \text{ mg L}^{-1}$ , while limonene and thymol up to 50 mg  $L^{-1}$  and vanillin and eugenol up to 500 mg  $L^{-1}$  did not affect ammonia concentrations in the rumen (Castillejos et al., 2006). This clearly demonstrates the optimization of a dose for a particular type of EO components. These effects might also depend upon the types of protein meal present in the diet. Wallace et al. (2002) investigated the rate of degradation of different protein meals and colonisation of feedstuffs incubated in nylon bags by attached enzyme activity in the presence of EO. The EO had a significant effect only on the breakdown of pea meal, the most rapidly degraded meal, of the protein meals tested. Bacterial proteinase and amylase associated with plants protein (pea, rapeseed, etc.) supplement tended to be lower in animals receiving EO, while corresponding activities associated with fishmeal were unaffected. Total microbial colonization associated with grass hay suspended in the bovine rumen was decreased by EO, while colonization of the less degradable fibrous substrates such as grass silage and barley straw was unaffected. This indicated that EO might suppress the colonization and/or digestion of readily degradable substrates by amylolytic and proteolytic bacteria without affecting fibre digestion (Wallace et al., 2002). A lack of effect of EO on rumen fermentation, particularly for in vivo studies may also involve adaptation of ruminal micro-organisms and the rapid metabolism of EO in the rumen to a less active form.

Methane production: Some components of EO have been tested for their inhibitory effects on methanogenesis (Table 1). For example, Evans and Martin (2000) observed that thymol (0.4 g L<sup>-1</sup>), a main component of EO derived from Thymus and Origanum plants, was a strong inhibitor of methane *in vitro*, but acetate and propionate concentrations also decreased. In another study, EO from Origanum vulgare and its component, thymol caused a suppression of methane to the extent of 99% at 6 mM concentration (Macheboeuf *et al.*, 2008). Anethole at 20 mg L<sup>-1</sup> medium caused an inhibition of methane *in vitro* (Chaves *et al.*, 2008c).

There are some in vitro studies showing inhibitory properties of EO mixtures or extracts derived from spices and plants (Table 1). Juniper berry EO and cinnamon oil (Chaves et al., 2008c) and peppermint oil (Tatsouka et al., 2008; Agarwal et al., 2009) have shown to have strong inhibitory effect on methanogenesis. The active component of cinnamon oil i.e., cinnamaldehyde caused a depression of methane production to the extent of 94% at 5 mM (Macheboeuf et al., 2008). The peppermint oil is known to contain menthol, menthone and menthyl acetate, which have shown to have antimicrobial properties (McKay and Blumberg, 2006). Methanol and ethanol extracts of fennel seeds and clove buds inhibited in vitro methane production (Patra et al., 2010). Eucalyptus oil inhibited methane production up to 58% at 1.66 mL  $L^{-1}$  (Kumar et al., 2009), 90.3% at 2 mL  $L^{-1}$ (Sallam et al., 2009) and 70% at a dose of 0.33 g of α-cyclodextrin-eucalyptus oil complex (Tatsouka et al., 2008). Various components of eucalyptus oils such as cineole, terpinenol, α-pinene, p-cymene, aromadendrene and α-phellandrene have been identified (Bhatti et al., 2007). The component of this oil, p-cymene decreased methane by 29% at a concentration of 20 mg L<sup>-1</sup> (Chaves et al., 2008c), however, α-cyclodextrin cineole did not influence methane up to a concentration of 0.33 g L<sup>-1</sup> (Tatsouka et al., 2008). The in vivo study of Wang et al. (2009) showed that inclusion of 0.25 g day<sup>-1</sup> of EO mixture from oregano plants in the diet of sheep for 15 days

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Table 1: Effects of essential oils or plants rich in essential oils on *in vitro* methane production and associated fermentation in the rumen (Patra and Saxena, 2010)

		Methane					
Essential oils	Dosage	inhibitiona	Comments	References			
Anethole	20 mg L <sup>-1</sup>	13.3%¶	TVFA and A/P unaffected	Chaves et al. (2008a)			
Carvaerol	$1.5\ \mathrm{to}\ 5\ \mathrm{mM}$	-8.4 to 88.9% $\P$	VFA decreased linearly, A/P	Macheboeuf et al. (2008)			
			increased				
Ciunamaldehyde	$1\ { m to}\ 5\ { m mM}$	$0.93 \text{ to } 89.3\%\P$	VFA decreased linearly, A/Pincreased	Macheboeuf et al. (2008)			
Cinnamon oil	$250 \ {\rm mg \ L^{-1}}$	70.9%¶	TVFA unaffected; A/P increased	Chaves <i>et al.</i> (2008a)			
${\tt EO} \ from \ \textit{Anethum graveolens}$	$2.5$ to $25\mathrm{mM}$	-0.35 to 41.7% $\P$	VFA decreased linearly, A/P increased	Macheboeuf $et\ al.\ (2008)$			
EO from Cinnamomum verum	1 to 10 mM	$3.19  ext{ to } 96.4\% \P$	VFA decreased linearly, A/P increased	Macheboeuf et al. (2008)			
EO mixture†	$1~{ m g~day^{-1}}$	-12.4%	DIG decreased; no effect on the rumen	Beanchemin and			
			fermentation	McGinn (2006)			
EO of oregano plant‡	$250~\mathrm{mg~day^{-1}}$	17.4%	DIG and A/P unaffected, TVFA increased	Wang et al. (2009)			
EO from Origanum vulgare	1 to 5 mM	-18.8 to $78.6\%\P$	VFA decreased linearly, A/P increased	Macheboeuf et al. (2008)			
Eucalyptus oil	0.33 to	30.3 to 78.3%	DIG unaffected; PROT decreased	Sallam <i>et al</i> . (2009)			
	$2ml\;L^{-1}$						
Eucalyptus oil	0.33 to	4.68 to 43.4%	TVFA decreased at higher	Kumar et al. (2009)			
	$1.66~{ m ml}~{ m L}^{-1}$		concentratious; PROT decreased;				
			A/P unaffected				
$Foeniculum\ vulgare\ {\rm seed}$	Methanol and	$3.47$ and $61.0\%\P$	DIG decreased with methanol extract,	Patra et al. (2010)			
extracts	ethanol extracts	\$	TVFA and A/P unaffected, PROT				
	of 0.5 mL/		decreased				
	30 mL						
Jumiper berry oil	$20~\rm mg~L^{-1}$	50.2%¶	TVFA and A/P unaffected	Chaves <i>et al.</i> (2008a)			
p-Cymene	$20~{ m mg~L^{-1}}$	29.1%¶	TVFA and A/P unaffected	Chaves <i>et al.</i> (2008a)			
Peppermint oils	0.33 to	19.9 to 75.6%	DIG and TVFA decreased; A/P ratio	Agarwal <i>et al.</i> (2009)			
	$2\mathrm{ml}\mathrm{L}^{-1}$		increased; PROT decreased				
Pinus mugos oil	$0.008~{ m g}~{ m L}^{-1}$	1.90%	DIG, TVFA, A/P and PROT unaffected	Soliv a <i>et al</i> . (2008)			
Syzygium aromaticumx	Methanol and	$15.5$ and $71.9\%\P$	DIG decreased, TVFA and A/P	Patra <i>et al</i> . (2010)			
	ethanol extracts	S	unaffected, PROT decreased				
	of 0.5 mL						
	30 mL						
Thymol	1 to 6 mM	-11.1 to 92.1% $\P$	VFA decreased linearly, A/P increased	Macheboeuf et al. (2008)			
Thymol (Thymus vulgaris)	0.5 to 3 mM	-4.43 to 66.1%¶	VFA decreased linearly, A/P increased	Macheboeuf et al. (2008)			
Thymol (Origanum vulgare)	0.5 to 3 mM	-19.6 to 68.1%¶	VFA decreased linearly, A/P increased	Macheboeuf et al. (2008)			

TVFA: Total volatile fatty acids concentration, DM: Dry matter; A/P: Acetate to propionate ratio, EO: Essential oils, DIG: Digestibility; PROT: Protozoal number decreased. Inhibition of methane production compared with control (without phytochemicals) relative to dry matter/organic matter degraded/digested unless otherwise marked. Relative to percentage of total gas. Study was in beef cattle; Study was in sheep

lowered methane. However, in vivo study of Beauchemin and McGinn (2006) with EO mixture fed to beef cattle (1 g day<sup>-1</sup>) for 21 days did not reveal any effect on methanogenesis. Similarly, Pinus mugos oil, which has been reported to contain pinene, limonellen,  $\omega$ 3-caren and  $\beta$ -phellandren with proportions of 35, 12, 25 and 14%, respectively, showed no antimethanogenic activity (Soliva *et al.*, 2008) probably due to inclusion of a low dose (8 mg L<sup>-1</sup>).

The EO exhibits different response on methanogenesis depending upon the type of EO. Macheboeuf *et al.* (2008) studied in detail on the dose-response effects of different EO on

methane inhibition and VFA production. The EO mixture extracted from Anethum graveolens (32% limonene) linearly decreased methane production. Cinnamaldehyde and a cinnamaldehyde-containing extract from Cinnamomum verum exhibited a negative sigmoidal shape response with a threshold dose of 3 mM, the concentration below which methane and VFA production were not altered. In the inflection point (4 mM) of the sigmoid curves for VFA production methane production completely inhibited. Carvacrol, thymol, EO extracts from Origanum vulgare (20% and 35% thymol) and Thymus vulgare (20% p-cymene) also showed a negative sigmoidal shape response, but had threshold doses of <2 mM and above this concentrations, a rapid decline in fermentation including methane were noted.

#### RUMINANT PERFORMANCE

Feed intake and growth: There are mixed observations on feed intake depending upon the type of EO and doses (Table 2). Feeding of 250 mg day<sup>-1</sup> of EO from oregano plants to sheep (Wang et al., 2009), 2 g of juniper berry EO (containing 35%  $\alpha$ -pinene) in cows (Yang et al., 2007), 0.75 or 2 g of a EO mixture to dairy cattle (Benchaar et al., 2006a, 2007a) and 0.043 g or

Table 2: Effects of essential oils (EO) on the production performance, digestibility and total volatile fatty acid (TVFA) concentrations in ruminants (% difference from control)

		$^{\dagger}$ Dosage	Feed	$\operatorname{ADG}\operatorname{or}$				
Essential oils	Species	$(g kg^{-1})$	intake	milk yield	FCR	Digestibility	TVFA	References
Carvacrol	Lamb	0.200	0.44	8.36	-8.60	-	19.0	Chaves <i>et al.</i> (2008a)
Carvacrol	Lamb	0.200	13.60	3.30	9.93	-	10.7	Chaves <i>et al.</i> (2008a)
Cinnamaldehyde	Lamb	0.200	-4.42	7.12	-11.9	-	13.7	Chaves <i>et al.</i> (2008a)
Cinnamaldehyde	Lamb	0.200	5.24	1.62	3.47	-	5.94	Chaves <i>et al.</i> (2008a)
Cinnamaldehyde	Lamb	0.200	4.62	15.4	-9.43	-	16.2	Chaves $et\ al.\ (2008b)$
Cinnamaldehyde	Steer	0.400	8.23	4.17	3.90	-	-	Yang et al. (2010a)
Cinnamaldehyde	Steer	0.400	10.30	-	-	2.57	2.72	Yang et al. (2010b)
Cinnamaldehyde	Steer	0.800	5.01	-1.79	6.92	-	-	Yang et al. (2010a)
Cinnamaldehyde	Steer	0.800	4.12	-	-	-2.19	1.50	Yang et al. (2010b)
Cinnamaldehyde	Steer	1.600	-1.16	-2.3	1.25	-	-	Yang et al. (2010a)
Cinnamaldehyde	Steer	1.600	-10.30	-	-	-6.55	3.07	Yang et al. (2010b)
EO from oregano	Sheep	0.250	-0.72	-	-	4.60	13.3	Wang et al. (2009)
EO mixture	Dairy goats	0.043	2.16	5.95	-3.58	-	-3.25	Malecky et al. (2009)
EO mixture	Dairy cows	0.320	-1.52	-2.20	0.69	0.81	-	Spanghero et al. (2008)
EO mixture	Dairy goats	0.430	-0.04	-1.17	1.15	-	-0.91	Malecky <i>et al.</i> (2009)
EO mixture	Dairy cows	0.640	-1.02	-0.73	1.39	1.88	-	Spanghero et al. (2008)
EO mixture	Dairy cows	0.750	-0.58	-5.12	4.79	-	5.10	Benchaar et al. (2007a)
EO mixture	Dairy cows	0.750	-1.13	-4.80	3.86	-	-10.3	Benchaar et al. (2007a)
EO mixture	Dairy cows	0.960	-0.51	-0.67	0.76	1.48	-	Spanghero et al. (2008)
EO mixture	Beef cattle	1.000	-2.41	-6.85	4.76	-7.29	-3.51	Beauchemin and
								McGinn, (2006)
EO mixture	Steer	1.000	-0.83	2.84	-3.57	2.78	14.7	Meyer et al. (2009)
EO mixture	Dairy cows	1.200	-7.35	-0.21	-7.15	-	-	Tassoul and Shaver (2009)
EO mixture	Dairy cows	2.000	-1.75	-6.69	5.29	1.21	-0.96	Benchaar et al. (2006a)
EO mixture	Dairy cows	32.000	-5.21	0.08	-5.21	-0.45	-	Santos et al. (2010)
Juniber berry EO	Dairy cows	2.000	-0.97	1.38	-2.31	0.88	-0.62	Yang et al. (2007)
Juniper berry EO	Lamb	0.200	5.12	17.3	-11.3	-	25.4	Chaves et al. (2008b)

†Dosage, g kg<sup>-1</sup> dry matter intake unless otherwise stated, ADG: Average daily gain, FCR: Feed conversion efficiency (kg of production/kg feed intake); DMI: Dry matter intake

0.43 g kg<sup>-1</sup> feed intake in dairy goats (Malecky et al., 2009) did not influence feed intake. However, an EO mixture of cinnamaldehyde (180 mg d<sup>-1</sup>) and eugenol (90 mg d<sup>-1</sup>) in beef cattle (Cardozo et al., 2006) and high doses of cinnamaldehyde (500 mg day<sup>-1</sup>) in dairy cattle (Busquet et al., 2003; cited by Calsamiglia et al., 2007) adversely affected feed intake. This reduction of intake might be related to palatability problems, suggesting that the product needs to be encapsulated to overcome this problem. In contrast, addition of capsicum oil (1 g day<sup>-1</sup> of capsicum extract containing 15% capsaicin) to a concentrate-based diet of beef cattle stimulated intake and rumen fermentation (Cardozo et al., 2006). Estell et al. (1998) studied the effect of some volatile compounds (camphor, limonene, cis-jasmone, β-caryophyllene, borneol and α-pinene) on the consumption of alfalfa pellets by sheep. Camphor, α-pinene and borneol depressed the consumption. The proper doses of EO supplementation is important because EO at low doses may stimulate intake whereas at higher doses may adversely affect intake in ruminants. Yang et al. (2010a) clearly demonstrated that cinnamaldehyde had greater feed intake response at low dose (0.4 g day<sup>-1</sup>), whereas, higher doses have no effect on intake (1.6 g day<sup>-1</sup>) in steers.

There is very limited information on effects of EO or their compounds on performances of ruminants. Bampidis et al. (2005) observed no change in Average Daily Gain (ADG) and feed were fed diets supplemented with oregano leaves when growing lambs (Origanum vulgare L.) providing 144 or 288 mg of oregano oil (850 mg g<sup>-1</sup> of carvacrol) per kilogram of diet DM. Benchaar et al. (2006b) noted no change in ADG of beef cattle fed a silage-based diet supplemented with 2 or 4 g day<sup>-1</sup> of a mixture of EO consisting of thymol, eugenol, vanillin and limonene. However, the EO mixture had a quadratic effect on feed conversion with the dose of 2 g day<sup>-1</sup> improving feed conversion compared with the dose of 4 g day<sup>-1</sup>. Chaves et al. (2008a) also reported that carvacrol or cinnamaldehyde (0.2 g kg<sup>-1</sup>) did not affect growth of sheep fed either corn-or barley-based diets for 11 weeks, although, growth was numerically higher in the barely-based diet compared with control (288 versus 310 g day<sup>-1</sup>). However, higher ADG (250 or 254 versus 217 g day<sup>-1</sup>) was observed when cinnamaldehyde or juniper berry EO was added to a barley-based diet at the similar concentration (0.2 g kg $^{-1}$  of dietary DM). Thus, it appears that the influence of EO on growth performance is diet-dependant.

Milk production and composition: The effect of EO on milk production is not consistent (Table 2). Santos et al. (2010) observed that feeding of EO mixture containing eugenol, geranyl acetate and coriander oil as major components increased the total yield of milk fat or fat percentage but has no effect on production of milk and other milk components. Increased fat synthesis might be due to enhanced acetate production and/or the ratio of acetate to propionate production in the rumen because of supplementation of EO (Benchaar et al., 2007b; Agarwal et al., 2009) or energetic shift away from body condition gain (Santos et al., 2010). Besides, there was a trend to a reduced DM intake with EO feeding without any effect on milk yield suggesting an improvement in efficiency of the utilization of nutrient (Santos et al., 2010). Although, the yields of milk and milk fat were not changed by the feeding of EO in the study of Tassoul and Shaver (2009), efficiency of milk production increased due to addition of EO in the diet of dairy cattle. In contrast, Kung et al. (2008) reported that dietary supplementation with EO (Crina) mixture increased milk yield.

There are some studies on EO in showing modification of the bio-hydrogenation process of fat in the rumen. Cinnamaldehyde, a component of EO, supplying at 500 mg L<sup>-1</sup> in a dual-flow

continuous culture fermenter affected the process of bio-hydrogenation of polyunsaturated fatty acids (Lourenço et al., 2008). Supplementation with cinnamaldehyde in that study inhibited the apparent bio-hydrogenation of C18:2 (linoleic acid) and C18:3 (linolenic acid) as reflected by the accumulation of intermediates such as trans-10 C18:1, trans-10, cis-12 C18:2 and trans-11, cis-15 C18:2. While, in vitro studies showed modifications of fatty acid profile in the rumen, the effect on milk fatty acid profile with the addition of cinnamaldehyde (1 g day<sup>-1</sup>) to the diet of dairy cattle (Benchaar and Chouinard, 2009) and a monoterpene blend (0.43 g kg<sup>-1</sup> diet) consisting of linalool, p-cymene,  $\alpha$ -pinene and  $\beta$ -pinene (45.2, 36.7, 16.0 and 2.2 mol/100 mol, respectively) to dairy goats (Malecky et al., 2009) was not always observed. Similarly, fatty acid profile of milk of cows supplemented daily with 750 mg of a mixture of EO compounds was not changed (Benchaar et al., 2007a). However, supplementing the same mixture at a higher concentration (i.e., 2 g day<sup>-1</sup>) increased the concentrations of conjugated linoleic acid (cis-9, trans-11 18:2.) in milk fat (Benchaar et al., 2006a).

The EO or their metabolic products could be present in milk and meat products due to feeding of EO. For example, feeding of caraway seed and camomile to goat resulted in limonene and carvone (the main EO component of caraway seed) in milk; however, the EO compounds present in camomile were not detected in milk (Molnar et al., 1997). Various monoterpenes such as  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -mircene, sabinene, camphene,  $\delta$ 3-carene and limonene were present in milk of cows grazing dicotyledons predominated Alpine pasture (Noni and Battelli, 2008; Chion et al., 2010). Therefore, the presence of EO or their derivatives could enrich specific organoleptic and nutritional properties to the dairy products that could provide an added value to the product (Chion et al., 2010).

#### CONCLUSIONS

In the recent decade, several studies have been conducted to exploit EO as feed additives for increasing the efficiency of ruminant production. Definitely, some EO constituents have favourable effects on rumen fermentation and ruminant production, which needs to identify optimising the dose, physiological status and feeding system. The EO can specifically inhibit HAP bacteria, methanogens and other undesirable bacteria, which might modulate rumen fermentation favourably such as increased concentrations of VFA in the rumen, inhibition of methane, decreased concentrations of ammonia and increased CLA production. Most of the findings are based on in vitro studies. A very few studies have been conducted in vivo and these results are not consistent because of different types and dose of EO components tested. Several thousands of active ingredients of EO have been identified, which might have different mode and range of actions in the rumen. The most of the studies used EO mixture with different active principles and proportions. An optimum dose of bioactive EO compounds and their appropriate combinations along with the dietary nutrient composition should be standardized to improve the efficiency of nutrient utilization and animal performance consistently. The adaptation of micro-organisms to EO compounds and their metabolism in the rumen have not been studied in details, which should be considered for exploitation of EO in ruminant nutrition.

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