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A review of plant-derived essential oils in ruminant nutrition and production☆

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Abstract

Public concern over use of antibiotics in livestock production has increased in recent years because of their possible contribution to emergence of antibiotic resistant bacteria, and their transmission from livestock to humans. Accordingly, ruminant microbiologists and nutritionists have been exploring alternative methods of favorably altering ruminal metabolism to improve feed efficiency and animal productivity. Plant extracts contain secondary metabolites, such as essential oils (EO), that have antimicrobial properties that make them potential alternatives to antibiotics to manipulate microbial activity in the rumen. Essential oils are naturally occurring volatile components responsible for giving plants and spices their characteristic essence and color. Over the last few years, a number of studies have examined effects of EO, and their active components, on rumen microbial fermentation. However, many of these studies are laboratory based (*i.e.*, *in vitro*) and of a short-term nature. Nevertheless, results from *in vitro* batch culture studies provide evidence that EO and their components have the potential to improve N and/or energy utilization in ruminants. Effects of EO on

Abbreviations: AA, amino acid; DM, dry matter; EO, essential oil; HAP, hyper-ammonia producing bacteria; MEO, mixture of essential oil compounds; VFA, volatile fatty acid

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ruminal N metabolism is more likely mediated by their impact on hyper-ammonia producing (HAP) bacteria resulting in reduced deamination of amino acids (AA) and production of ammonia N. However, these responses are only observed with high doses of EO, which also can inhibit the process of ruminal fermentation as reflected by a decline in total volatile fatty acid production. Effects on methane production are inconsistent, but evidence to date indicates that there is potential to select EO, or active components, that selectively inhibit ruminal methanogenesis. Results from *in vitro* continuous culture studies suggest that rumen microbial populations may adapt to EO, which may explain the lack of an effect of EO on ruminal metabolism and animal performance in long-term *in vivo* studies. Several studies have examined the activity of a number of EO against a wide variety of food-borne pathogens. Data available show a strong bactericidal activity against pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella* spp. Essential oils hold promise as feed additives in ruminant nutrition to improve feed efficiency and control the spread of pathogens in livestock. However identification of EO, or their active components, that favorably alter fermentation without resulting in broad overall inhibition of rumen fermentation, continues to be a major challenge for researchers.

Keywords: Essential oil; Ruminant; Metabolism; Production; Control of pathogens

1. Introduction

In livestock production systems, antibiotics are commonly fed to animals to prevent disease and metabolic disorders, as well as improve feed efficiency. However in recent years, public concern over routine use of antibiotics in livestock nutrition has increased due to the emergence of antibiotic resistant bacteria that may represent a risk to human health. Consequently, considerable effort has been devoted towards developing alternatives to antibiotics. Plant extracts offer a unique opportunity in this regard (Wallace, 2004), as many plants produce secondary metabolites, such as saponins and tannins, which have antimicrobial properties. These compounds have been shown to modulate ruminal fermentation to improve nutrient utilization in ruminants (Wang et al., 1996; Hristov et al., 1999). Similarly, the well documented antimicrobial activity of essential oils (EO), and their active components, has prompted a number of scientists to examine the potential of these secondary metabolites to manipulate rumen microbial fermentation to improve production efficiency in ruminants. Contrary to their name, EO are not true oils (*i.e.*, lipids) and are commonly derived from the components responsible for fragrance, or *Quinta essentia*, of plants. Essential oils are considered safe for human and animal consumption, and are categorized as generally recognized as safe (GRAS; FDA, 2004) in the USA.

The antimicrobial properties of EO have been demonstrated against a wide range of microorganisms, including bacteria, protozoa and, fungi (Dean and Ritchie, 1987; Sivropoulou et al., 1996; Chao et al., 2000). Essential oils have also been exploited for their activity against a wide variety of food-borne pathogens. For example, *Escherichia coli* O157:H7 was inhibited by oregano oil and its two main components carvacrol and thymol (Helander et al., 1998; Elgayyar et al., 2001).

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Table 1
Concentrations of carvacrol, thymol, *p*-cymene, and γ-terpinene in thyme essential oils (adapted from Martínez et al., 2006)

Component (%)	Source of thyme essential oils	
	Thymus hyemalis Lange	*Thymus zygis* subsp. *gracilis*
Carvacrol	24.3	3.13
Thymol	4.79	62.1
p-Cymene	20.93	17.03
γ-Terpinene	18.0	3.13

This review discusses recent developments in use of EO to potentially benefit ruminant production. Mechanisms of action are discussed, including effects on ruminal microbial populations, ruminal fermentation, animal performance, and control of pathogens.

2. Definition and chemistry

Essential oils, also known as volatile or ethereal oils, occur in edible, medicinal, and herbal plants. As these aromatic compounds are largely volatile, they are commonly extracted by steam distillation or solvent extraction (Simon, 1990; Greathead, 2003). Essential oils can be extracted from many parts of a plant, including the leaves, flowers, stem, seeds, roots and bark. However, the composition of the EO can vary among different parts of the same plant (Dorman and Deans, 2000). For instance, EO obtained from the seeds of coriander (*Coriandrum sativum* L.) have a different composition from the EO of cilantro, which is obtained from the immature leaves of the same plant (Delaquis et al., 2002). Chemical differences among EO extracted from individual plants, or different varieties of plants, also exist and are attributed to genetically determined properties, age of the plant, and the environment in which the plant grows (Cosentino et al., 1999). For instance, Martínez et al. (2006) observed that the concentration of carvacrol, thymol, *p*-cymene and γ-terpinene in thyme EO varied widely depending on the species of the thyme plant (Table 1). Chemically, EO are variable mixtures of principally terpenoids, mainly monoterpenes (C_{10}) and sesquiterpenes (C_{15}), although diterpenes (C_{20}) may also be present, and a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally N- and S-containing compounds, coumarins and homologues of phenylpropanoids (Dorman and Deans, 2000). The major chemical components of some common EO are in Table 2 and examples of the chemical structures of some EO are in Fig. 1.

3. Antimicrobial properties

Plant secondary metabolites have traditionally held an important role in human health and wellness (Bode and Müller, 2003). Exploited for their essence, flavor, antiseptic and/or preservative properties, plants and their extracts have been used by mankind since early history (Burt, 2004). Early accounts of plant-based, traditional medicine date back to

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Table 2
Examples of some essential oils and their main components (adapted from Chao et al., 2000)

Essential oil	Plant part	Botanical source	Main components	% of total
Angelica	Roots	*Angelica archangelica* L.	α-Pinene	24.7
			δ-3-Carene	10.5
			α-Phellandrene + myrcene	10.8
			limonene	12.9
			β-Phellandrene	10.4
			p-Cymene	7.7
Bergamot	Fruits	*Citrus bergamia* Risso et Poit	β-Pinene	7.7
			Limonene + β-phellandrene	39.4
			γ-Terpinene	8.6
			Linalool	11.1
			Linalyl acetate	28.0
Cinnamon	Inner bark	*Cinnamomum zeylanicum* Blu.	(*E*)-Cinnamaldehyde	77.1
			Eugenol	7.2
Coriander	Seeds	*Coriandrum sativum* L.	*p*-Cymene	6.1
			Linalool	72.0
Dill (Indian)	Seeds	*Anethum sowa Roxb*	Limonene	50.9
			trans-Dihydrocarvone	10.4
			Carvone	20.3
			Dillapiole	36.6
Eucalyptus	Leaves	*Eucalyptus citriodora* K. D. Hill	Citronellal	72.8
			Citronellol	14.5
Ginger	Roots	*Zingiber officinale* Rosc.	Camphene	14.1
			Neral	4.9
			Geranial + bornyl acetate	8.1
			β-Bisabolene	22.1
			ar-Curcumene	14.5
			β-Eudesmol	5.4
Juniper	Berries	*Juniperus communis* L.	α-Pinene	33.7
			Sabinene	27.6
			Myrcene	5.5
Orange	Peel	*Citrus sinensis L.* Osbeck	Limonene	91.5
Pepper	Fruits	*Piper nigrum L.*	α-Pinene	9.0
			β-Pinene	10.4
			Sabinene	19.4
			δ-3-Carene	5.4
			Limonene	17.5
			β-Caryophyllene	14.7
Rosemary	Whole plant	*Rosemarinus officinalis* L.	α-Pinene	7.4
			β-Pinene	5.0
			1,8-Cineole	43.6
			Camphor	12.3
Tea tree	Branches	*Melaleuca alternifolia* L.	α-Terpinene	10.4
			1,8-Cineole	5.1
			Terpinene-4-ol	40.1
			γ-Terpinene	23.0

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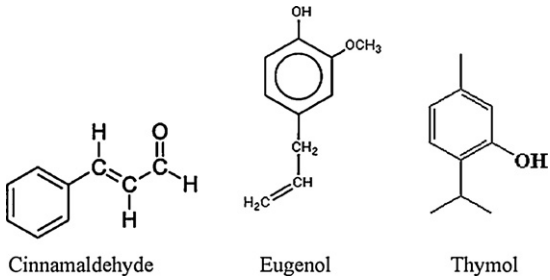

Fig. 1. Examples of chemical structures of some essential oil compounds.

Mesopotamia, approximately 2600 BC (Greathead, 2003). Plant-based medicine was widely practised until the early 20th, century at which point it was rapidly phased out due to the introduction of novel and effective synthetic medicines that could be produced more economically and had easily identifiable benefits to human health (Greathead, 2003). In recent years however, the emergence of multi-drug resistant bacteria, and the risk it represents to human health, has renewed interest in plant extracts.

Antimicrobial activities of EO have been demonstrated against a wide variety of microorganisms, including Gram-positive and Gram-negative bacteria. The antimicrobial activity of EO has been attributed to a number of terpenoid and phenolic compounds (Panizzi et al., 1993; Helander et al., 1998; Chao et al., 2000), as well as the chemical constituents and functional groups contained in the EO, the proportions in which they are present and the interactions between them (Dorman and Deans, 2000). Additive, antagonistic, and synergistic effects have been observed between components of EO (Burt, 2004). By assessing the minimum inhibitory concentration of oregano EO and its two main constituents, thymol and carvacrol, against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, Lambert et al. (2001) observed that the combination of thymol and carvacrol exhibited higher antibacterial activity than either compound alone and that the inhibitory effect of oregano EO is mainly due to the additive antibacterial action of these two compounds. Delaquis et al. (2002) examined the antibacterial activity of crude oils and the distilled fractions of dill (*Anethum graveolens* L.), coriander (seeds of *Coriandrum sativum* L.), cilantro (leaves of immature *C. sativum* L.), and eucalyptus (*Eucalyptus dives*) against some common Gram-positive and Gram-negative food spoilage bacteria (*i.e.*, *Salmonella typhimurium*, *Listeria monocytogenes*, *S. aureus*, *P. fragi*, *Serratia grimesii*, *Enterobacter agglomerans*, *Yersinia enterocolitica*, *Bacillus cereus*). Results showed that the magnitude and the spectrum of antibacterial activity of these individual fractions frequently exceeded those of the crude oils. For instance, crude dill EO had weak antimicrobial activity, while distilled fractions of dill EO contained higher concentrations of the main chemical constituents, D-limonene and carvone, and exhibited higher antimicrobial activity. Mixing distilled fractions of cilantro and eucalyptus resulted in additive, synergistic, and antagonistic effects against the species of bacteria examined. Mourey and Canillac (2002) evaluated the antibacterial activity of six main components of conifer EO (*i.e.*, α- and β-pinene, *R*- and *S*-limonene, 1,8 cineole, and borneol) against the bacterium *L. monocytogenes*. With the exception 1,8 cineole, all individual components had higher bacteriostatic activity than fir or pine oil (Canillac

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and Mourey, 1996), suggesting that other components within whole oil reduce, or dilute, the antimicrobial activity of the individual components. In contrast, a comparison of the minimum inhibitory concentration of individual components with that of whole spruce oil (Canillac and Mourey, 2001) showed that individual components were less active, or similar in activity, than whole spruce oil suggesting that the antimicrobial activity of spruce EO is the result of the synergistic effects between individual components contained in the EO.

4. Mode of action

A number of theories have been proposed to explain the mechanism by which EO exert antibacterial activity. Given that EO comprise a large number of components, it is most likely that their antibacterial activity is not due to one specific mode of action but involves several targets in the bacterial cell (Skandamis et al., 2001; Carson et al., 2002; Burt, 2004). Acamovic and Brooker (2005) suggested that because plant secondary metabolites, including EO, interact with a wide variety of cellular components and can modulate a response at their targets, these compounds have the ability to modulate a large number of cellular targets. It is believed that most EO exert their antimicrobial activities by interacting with processes associated with the bacterial cell membrane, including electron transport, ion gradients, protein translocation, phosphorylation, and other enzyme-dependent reactions (Ultee et al., 1999; Dorman and Deans, 2000). Helander et al. (1998) showed that thymol from thyme oil (*Thymus vulgaris*) and carvacrol from oregano oil (*Origanum vulgaris*) both disrupt the cell membrane thereby decreasing the intracellular ATP pool and increasing the extracellular ATP pool in *E. coli*.

Essential oils have a high affinity for lipids of bacterial cell membranes due to their hydrophobic nature, and their antibacterial properties are evidently associated with their lipophilic character. Dorman and Deans (2000) noted that this mechanism is a function of the lipophilic properties of the constituent component of EO and the potency of their functional group. Burt (2004) suggested that Gram-positive bacteria appear to be more susceptible to the antibacterial properties of plant EO compounds than Gram-negative bacteria. This may be expected as Gram-negative bacteria have an outer layer surrounding their cell wall that acts as a permeability barrier, limiting the access of hydrophobic compounds. However, Helander et al. (1998) reported that the phenolics thymol and caravacrol also inhibited growth of Gram-negative bacteria by disrupting the outer cell membrane. It appears that the small molecular weight of EO allows them to penetrate the inner membrane of Gram-negative bacteria (Nikaido, 1994; Dorman and Deans, 2000). Trombetta et al. (2005) reported that the monoterpenes linalyl acetate, menthol, and thymol were active against Gram-positive *Staphlococcus aureus* and Gram-negative *E. coli*., and suggested that antimicrobial effect of these monoterpenes is due to the disruption of the plasma membrane of bacteria, thereby interfering with membrane permeability causing intracellular leakage.

5. Effects on rumen microbial fermentation

The well-documented antimicrobial activity of EO has recently prompted a number of researchers to examine their potential to manipulate ruminal fermentation as a means of

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improving feed efficiency and nutrient utilization by ruminants. The limited number of EO and EO compounds evaluated to date show some promise in this regard. However, the range of EO and their component compounds available is extensive and many of them have yet to be examined for this purpose. Furthermore, most studies have been conducted *in vitro* and research is needed to determine effects *in vivo*, the mode of action of the various EO and their compounds and the concentrations that favorably modify ruminal fermentation.

Various studies have been conducted to determine effects of EO and their components on rumen microbial fermentation. These studies used a wide range of EO and EO compounds, dose rates and diets and, not surprisingly, results have been inconsistent. The varied response among EO products evidently reflects differences in chemical structure, which influences their effects on microbial activity.

Initially, ruminant nutritionists were interested in EO mainly because of their role in reducing the palatability of some plant species. Oh et al. (1967, 1968) and Nagy and Tengerdy (1968) were the first to investigate effects of EO on ruminal microbial fermentation, as gas production, *in vitro*. Nagy and Tengerdy (1968) observed that EO extracted from Sagebrush (*Artemesia tridentata*) markedly inhibited activity of ruminal bacteria *in vitro*. Oh et al. (1967) showed that EO extracted from Douglas fir needles (*Pseudotsuga menziesii*) exerted a general inhibitory effect on ruminal bacteria activity *in vitro*. The degree of inhibition depended, however, on the chemical structure of the EO compound added. Of the compounds evaluated, oxygenated monoterpenes, particularly monoterpene alcohols and aldehydes, strongly inhibited growth and metabolism of rumen microbes, whereas monoterpene hydrocarbons slightly inhibited and, sometimes, stimulated activity of rumen microbes. These findings were some of the first to demonstrate that the chemical composition of EO greatly influences their effects on activity of ruminal microorganisms. Much of the current research with EO in ruminant nutrition has centered upon their potential to improve ruminal N and energy utilization.

5.1. *Effects on protein metabolism*

Symbiosis between ruminants and their microflora instils ruminants with the unique advantage of being able to utilize non-protein sources of N as nutrients. The microbial protein that flows from the rumen to the small intestine provides the host with an excellent source of amino acids (AA) for synthesis of milk and meat proteins. However, the microbial proteins synthesized in the rumen are not sufficient to support the AA requirements of high-producing ruminants. Consequently, diets are usually supplemented with sources of feed protein, but such practices can increase feed costs. Furthermore, inefficient N utilization by ruminants results in excretion of N-rich wastes to the environment. Lapierre et al. (2005) estimated that about 0.3 of the N consumed by the dairy cow is excreted in urine. Therefore, improving N utilization has a positive impact on efficiency of animal production and on the environment.

Several *in vitro* (batch or continuous cultures) studies have been conducted to determine effects of EO and their components on ruminal N metabolism. Early work by Borchers (1965) showed that the addition of thymol to ruminal fluid (1 g/l) containing casein resulted in an accumulation of AA and a decrease in ammonia N (NH_3–N) concentration, suggesting inhibition of AA deamination by ruminal bacteria. Broderick and Balthrop (1979) also

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Table 3
Effect of feeding a mixture of essential oil compounds to dairy cows (1 g/day) on the subsequent breakdown of proteins, peptides, and amino acids in rumen fluid in vitro (adapted from McIntosh et al., 2003)

	Control	EO compounds mixture[a]	SED
Amount (mg) of substrate degraded/mg of protein/h			
Casein	0.46	0.49	0.038
Lactoglobulin	0.21	0.24	0.032
Hide powder azure	0.048	0.049	0.0035
Albumin	0.033	0.035	0.0016
Elastin congo red	0.0056	0.0054	0.00092
Amount (nmol) of peptide degraded mg/of protein/h			
Ala_2	0.60	0.69	0.088
Ala_5	1.03	1.22	0.230
Arg, Lys, Asp, Val, Tyr	0.33	0.35	0.035
$Ala_{2-p}NA$	1.64	1.58	0.056
GlyArg-MNA	0.36	0.40	0.060
Amount(nmol) of NH_3 produced/mg of protein/h			
No monensin	410	372	9.8[b]
5 μM monensin	280	287	10.9

[a] Essential oil compounds mixture (Crina® ruminants; Akzo Nobel Surface Chemistry Ltd., Hertfordshire, UK).
[b] $P<0.05$.

observed that thymol inhibited deamination of AA to NH_3–N. More recently, McIntosh et al. (2003) observed a 9% reduction in the rate of AA deamination when casein acid hydrolysate was incubated *in vitro* for 48 h in batch cultures of ruminal fluid collected from cows fed a silage-based diet supplemented with 1 g/day of a commercial mixture of EO compounds (MEO; Crina® ruminants; Akzo Surface Chemistry Ltd., Herfordshire, UK), (Table 3). The Crina® supplement contains 100–300 g/kg of phenolic compounds including cresol, resorcinol, thymol, guaiacol and eugenol (Rossi, 1994). Newbold et al. (2004) also reported a reduction, −24%, in the *in vitro* rate of AA deamination when casein acid hydrolysate was incubated for 24 h with ruminal fluid collected from sheep fed diets containing 110 mg of MEO (Table 4). In both studies, peptidolytic and proteolytic activities in ruminal fluid were unaffected by MEO. In the same study, McIntosh et al.

Table 4
Proteolytic, peptidolytic and deaminative activities of ruminal fluid collected from sheep fed a silage-based diet supplemented with 110 mg/day of a mixture of essential oil compounds (adapted from Newbold et al., 2004)

	Control	EO compounds mixture[a]	SED
Proteolytic activity (mg ^{14}C casein degraded/mg protein/h)	1.40	1.43	0.045
Peptidolytic activity			
Hydrolysis of Ala_2 (mmol/mg of protein/min)	1.19	1.06	0.173
Hydrolysis of Ala_5 (mmol/mg of protein/min)	2.62	2.71	0.171
Deaminase acitivity (nmol NH_3 produced/mg of protein/h)	204	155	9.27[b]

[a] Essential oil compounds mixture (Crina® ruminants; Akzo Nobel Surface Chemistry Ltd., Hertfordshire, UK).
[b] $P<0.05$.

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(2003) observed no additional reduction in the rate of deamination when the ionophore monensin was added to ruminal fluid, suggesting that the bacterial species affected by MEO were the same as those inhibited by monensin. In further work, McIntosh et al. (2003) demonstrated that MEO inhibited growth of some (*i.e.*, *Clostridium sticklandii* and *Peptostreptococcus anaerobius*) hyper-ammonia producing (HAP) bacteria, but other HAP bacteria (e.g., *Clostridium aminophilum*) were less sensitive. Hyper-ammonia producing bacteria are present in low numbers in the rumen (<0.01 of the rumen bacterial population), but they possess a very high deamination activity (Russell et al., 1988). Wallace (2004) reported that the number of HAP bacteria was reduced by 77% in sheep receiving a low-protein diet supplemented with MEO at 100 mg/day, but that MEO had no effect on HAP bacteria when sheep were fed a high-protein diet. Collectively, results of the studies by McIntosh et al. (2003), Newbold et al. (2004), and Wallace (2004) suggest that effects of EO on ruminal protein metabolism are on AA degradation (*i.e.*, deamination) and these effects are likely due to inhibition of HAP bacteria.

Others have used continuous culture systems to explore effects of EO and their constituents on ruminal N metabolism. Using dual-flow continuous culture fermenters for 8 days of incubation, and maintained at constant pH, Castillejos et al. (2005) observed that addition of MEO at 1.5 mg/l had no effect on NH_3–N concentration, bacterial and dietary N flows, degradation of crude protein, or efficiency of microbial protein synthesis. The lack of effect of MEO on N metabolism was attributed to the dose of 1.5 mg/l, which may have been too low to alter activity of ruminal bacteria. However, when Castillejos et al. (2007) used the same MEO at higher concentrations (*i.e.*, 5, 50, and 500 mg/l) there was still no effect of MEO on ruminal N metabolism (*i.e.*, ruminal concentrations of NH_3–N, small peptides + AA, and large peptides) in continuous fermenters for 9 days of incubation maintained at constant pH. McIntosh et al. (2003) and Newbold et al. (2006) suggested that concentrations of MEO above 35 mg/l would be required to substantively alter N metabolism in the rumen, a level that may be difficult to achieve *in vivo*. Indeed, Benchaar et al. (2006b, 2007) observed no change in ruminal NH_3–N concentration, N retention, and N digestibility when lactating dairy cows were supplemented with MEO at doses of 0.75 or 2 g/day. Assuming a rumen volume of 100 litres and an outflow rate of 0.1/h for an adult dairy cow, ruminal concentration of MEO would have been 3.1 and 8.3 mg/l for each of the doses, respectively. These ruminal concentrations are indeed much lower than the range of concentrations (*i.e.*, 35–360 mg/l) required for MEO to alter N metabolism of ruminal bacteria (McIntosh et al., 2003). However, McIntosh et al. (2003) speculated that local concentrations of EO, some of which are often sparingly soluble, may be higher on the surface of ingested plant materials, which may increase the bactericidal effects of EO *in vivo*.

Other studies used the rumen *in situ* bag technique to investigate effects of MEO on ruminal N metabolism. For example, Molero et al. (2004) used growing heifers to evaluate the influence of MEO (700 mg/day) on *in situ* ruminal degradability of proteins in soybean meal, corn gluten feed, fish meal, green peas, sunflower meal, and lupin seeds. Of the five protein supplements examined, MEO only tended to reduce the effective ruminal protein degradabilities of lupin seeds, green peas, and soybean meal, and the reductions were too small to have any likely nutritional impact on ruminal protein metabolism in the animal. Recent studies by Newbold et al. (2004) and Benchaar et al. (2006b) reported no change in

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the kinetics of protein degradation from soybean meal incubated in the rumen of sheep or dairy cows supplemented daily with 110 mg or 2 g of MEO, respectively.

The lack of effects of MEO on N metabolism in long-term (*i.e.*, continuous culture) *in vitro*, ruminal *in situ*, and *in vivo* studies compared to short-term *in vitro* batch culture studies may be related to the duration that ruminal bacteria are exposed to EO. Longer duration of exposure may result in shifts in microbial populations, and it is possible that some of the EO compounds are subject to degradation by ruminal bacteria. Cardozo et al. (2004) and Busquet et al. (2005c) observed that some of the effects of EO and their main components on rumen microbial fermentation dissipated after 6–7 days of fermentation in a dual flow continuous-culture system, suggesting that rumen microbial populations may adapt to EO. Therefore, results from *in vitro* batch culture studies must be interpreted with caution as they report effects over a set incubation time (*e.g.*, 24 or 48 h) and do not account for possible shifts in microbial populates that may occur as a result of exposure of rumen microbes to EO.

More recently, a number of studies have shown that factors such as the chemical composition and dosage rate of EO could influence effects of EO on ruminal N metabolism. Busquet et al. (2005c) observed that the addition of clove bud EO (*Syzygium aromaticum*) to a continuous culture fermenter at 2.2 mg/l strongly reduced (*i.e.*, −80%) the concentration of large peptides, but had no effect on NH_3–N concentration, suggesting that clove bud EO reduced the peptidolytic activity of ruminal bacteria. However, addition of the principal component of clove bud EO, eugenol, at the same concentration had no effect on N metabolism, suggesting that the anti-peptidolytic activity of clove bud EO is not due to its main component, but results from unidentified compounds within the oil fraction. In contrast, Busquet et al. (2006) reported that, when supplied at the same concentration (*i.e.*, 3000 mg/l), both oregano EO and its major constituent carvacrol reduced the concentration of NH_3–N in *in vitro* batch cultures, indicating that carvacrol accounts for the majority of the antimicrobial activity in oregano EO.

The chemical composition of EO may also affect the manner in which they alter ruminal N metabolism. Castillejos et al. (2006) observed variation in the effects of increasing dosage levels (5, 50, 500, and 5,000 mg/l) of different EO compounds on fermentation products in 24 h *in vitro* batch cultures of rumen fluid. The aldehyde vanillin was ineffective at altering NH_3–N concentration at doses of 5, 50 and 500 mg/l while the monoterpene limonene reduced NH_3–N concentration at the dose of 500 mg/l. The phenolic eugenol decreased NH_3–N concentration at concentrations of 5, 50, and 500 mg/l, whereas the phenolic guaiacol decreased NH_3–N concentration at all concentrations. These results illustrate that effects of EO components on N metabolism vary with chemical structure. In general, phenolic compounds have been shown to have high antimicrobial activity due to the presence of a hydroxyl groups within the phenolic structure (Dorman and Deans, 2000; Ultee et al., 2002; Burt, 2004). In a study investigating the antibacterial properties in berries, Puupponen-Pimiä et al. (2001) determined that the phenolic compounds cinnamic acid, 3-coumaric acid, caffeic acid, and ferulic acid extracted from various berries (*i.e.*, blueberry, cranberry, raspberry, strawberry, and black currants) inhibited Gram-negative bacteria, but were ineffective in inhibiting Gram-positive bacteria. Compounds with phenolic structures have a broad spectrum of activity against a variety of both Gram-positive and Gram-negative bacteria (Helander et al., 1998; Dorman and Deans, 2000; Lambert et al., 2001).

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Essential oils and their components have been shown to affect ruminal N metabolism in a dose-dependent manner. For instance, Busquet et al. (2006) demonstrated that some EO (*i.e.*, anise oil, cade oil, capsicum oil, cinnamon oil, clove, bud oil, dill oil, garlic oil, ginger oil, oregano, oil, and tea tree oil) and their main components (*i.e.*, anethol, benzyl salicylate, carvacrol, carvone, cinnamaldehyde, and eugenol) markedly inhibited NH_3–N concentration at high concentrations (*i.e.*, 3000 mg/l), but effects were marginal at moderate doses (*i.e.*, 300 mg/l) and nonexistent at low doses (*i.e.*, 3 mg/l). The decreased ruminal NH_3–N concentration was, however, associated with a reduction in total VFA concentration, suggesting a reduction in overall fermentation of the diet. Because VFA are the principal source of energy for ruminants, decreasing ruminal VFA production could have adverse nutritional consequences if this effect was expressed *in vivo*.

Information on effects of EO and their constituents on ruminal bacterial N escape are scarce. Flow of bacterial N was not changed by addition of garlic EO or cinnamaldehyde (Busquet et al., 2005a), but it was reduced by cinnamon leaf EO (Fraser et al., 2007) in continuous culture fermenters. Newbold et al. (2004) and Benchaar et al. (2006b) observed no change in duodenal bacterial N flow of sheep and dairy cows fed daily dosages of 110 mg and 2 g of MEO, respectively. The discrepancy between studies is probably due to the dose, chemical composition of EO, and experimental conditions.

Ruminal protozoa have a negative role on utilization of N by ruminants. Protozoa engulf and digest large numbers of ruminal bacteria thereby decreasing net microbial protein flow from the rumen to the duodenum (Ivan et al., 2000). Protozoa also possess proteolytic and deaminating activities (Williams and Coleman, 1992). Thus, removal of protozoa from the rumen (*i.e.*, defaunation) prevents recycling of N between bacteria and protozoa, which results in increased flow of microbial N from the rumen. For instance, bacterial protein flow to the intestine in defaunated sheep was 35% higher than the microbial protein flow in faunated sheep (Ivan et al., 1992). Increased bacterial protein synthesis in the rumen due to defaunation could benefit the host by supplying additional AA for absorption. Moreover, improved efficiency of N metabolism in the rumen could reduce N losses in feces and urine. Due to the lack of a suitable defaunating agent, and spontaneous refaunation, defaunation has not been practical in commercial ruminant production systems. Plant extracts, such as condensed tannins and steroidal saponins, have been extensively investigated for their inhibitory effects on ciliate ruminal protozoa (Wallace et al., 1994; Wang et al., 1996; Wang et al., 2000; Min et al., 2002). However, few studies to date have evaluated the effects of EO and their compounds on ruminal protozoa. Ando et al. (2003) reported that feeding 200 g/day (*i.e.*, 30 g/kg of total dietary DM) of peppermint (*Menthaxpiperita* L.) to Holstein steers decreased the total number of protozoa, and the numbers of *Entodinum*, *Isotrica*, and *Diplodium*. Mohammed et al. (2004) observed no change in the number of protozoa in ruminal fluid of steers supplemented with cyclodextrin encapsulated horseradish oil at 20 g/kg of dietary DM. McIntosh et al. (2003) observed that the bacteriolytic activity of rumen ciliate protozoa was unaffected in dairy cows supplemented with 1 g/day of MEO. Newbold et al. (2004) and Benchaar et al. (2007) reported that ruminal protozoa counts were not affected when sheep and dairy cows were fed 110 and 750 mg/day of MEO, respectively.

Supplementation of dairy cows diets with 1 g/day of cinnamaldehyde had no effect on the number, or generic composition, of ciliate protozoa (Benchaar et al., 2005). Cardozo et al. (2006) observed that addition of a mixture of cinnamaldehyde (180 mg/day) and eugnol

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(90 mg/day) to the diets of beef heifers increased numbers of holotrichs and had no effect on entodiniomorphs, but that there was no effect on numbers of these protozoal species when the mixture contained higher concentrations of cinnamaldehyde (600 mg/day) and eugenol (300 mg/day). In contrast, feeding 2 g/day of anise extract containing 100 g/kg of anethol to beef heifers decreased counts of holotrichs and entodiniomorphs (Cardozo et al., 2006). Overall, EO and their components have no marked effect on numbers and/or activity of ruminal ciliate protozoa.

5.2. *Effects on volatile fatty acid production*

Supplementation with EO or EO compounds has increased ruminal total VFA concentration, which may indicate improved feed digestion, in a limited number of studies. In one such study, addition of 1.5 mg/l of MEO increased total VFA concentration in continuous cultures maintained at constant pH, although there was no concomitant increase in organic matter digestibility (Castillejos et al., 2005). Two *in vivo* studies with MEO reported no effects when fed to sheep (110 mg/day) or cattle (1 g/day) on total VFA concentration or proportions (Newbold et al., 2004; Beauchemin and McGinn, 2006). It is possible that effects of MEO on total VFA concentration may depend on the composition of the diet. Benchaar et al. (2007) reported that MEO (750 mg/day) tended to increase total VFA concentration in the rumen of lactating cows when the diet contained alfalfa silage, but tended to decrease total VFA concentration when the diet contained corn silage. Mohammed et al. (2004) reported that increasing levels (*i.e.*, from 0.17 to 1.7 g/l) of cyclodextrin encapsulated horseradish linearly increased total VFA concentration in batch cultures. When the same product was fed to cattle, there was a very small increase in total VFA, but no change in feed digestibility.

Overall, supplementation with EO or their components has caused either a decrease or no change in total VFA concentration in most studies. Whether VFA concentration decreases as a result of the antimicrobial effects of EO may be dose dependent. For example, Busquet et al. (2006) studied effects of various plant extracts (*i.e.*, anise oil, cade oil, capsicum oil, cinnamon oil, clove, bud oil, dill oil, fenugreek, garlic oil, ginger oil, oregano, oil, tea tree oil, and yucca), and secondary plant metabolites (*i.e.*, anethol, benzyl salicylate, carvacrol, carvone, cinnamaldehyde, and eugenol) on ruminal fermentation in a 24 h batch culture. Each treatment was supplied at varying doses up to 3 g/l of culture fluid. None of the EO or EO compounds increased total VFA concentration but, at the highest concentration, most treatments decreased total VFA concentration, a possible reflection of decreased feed digestion. Similar effects were reported by Castillejos et al. (2006) for eugenol, guaiacol, limonene, thymol, and vanillin using doses up to 5 g/l. These EO compounds generally had no effect on total VFA concentration, with the exception of the highest dose, which decreased total VFA concentration in cultures for all compounds.

Lack of a change in total VFA concentration could be viewed as desirable if it was accompanied by changes such as decreased NH_3–N concentration, decreased methane production, or a change in molar proportions of VFA. However, a reduction in total VFA production as a result of EO supplementation would generally be viewed as nutritionally unfavorable. The challenge is to identify the dose rates for various EO or EO active components that favorably alter aspects of rumen metabolism without reducing total VFA concentrations.

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A number of studies have shown that certain EO and their components shift molar proportions of VFA in a manner similar to that of monensin (*i.e.*, decreased acetate and increased propionate proportions; McGuffey et al., 2001), which is viewed as a favorable outcome of EO supplementation. Mohammed et al. (2004) reported decreased acetate proportion, and increased propionate proportion, with cyclodextrin encapsulated horseradish both *in vitro* and *in vivo*. Busquet et al. (2005a) used cinnamaldehyde and garlic oil added at two doses (*i.e.*, 31.2 and 312 mg/l of culture fluid) in a continuous culture study. At the low dose of cinnamaldehyde, and the high dose of garlic oil, acetate proportion decreased and that of propionate increased. Molar proportion of butyrate also increased at the high dosage rate. Thus the EO, or the active components and dose rates used in that study, resulted in effects similar to monensin, with the exception of the increase in butyrate concentration. In a subsequent study by this group (Busquet et al., 2006), garlic oil (300 and 3000 mg/l) and benzyl salicylate (300 and 3000 mg/l) also reduced acetate and increased propionate and butyrate proportions. High butyrate concentration as a result of supplementation with some EO compounds suggests that the mode of action of these compounds differs from that of monensin.

While many studies have shown beneficial changes in VFA profiles, some EO produce undesirable changes in proportions of individual VFA. For example, Castillejos et al. (2006) reported that eugenol (500 mg/l) reduced the proportion of propionate, without affecting total VFA concentration. Cardozo et al. (2005) showed *in vitro* that effects of EO and their components on VFA profile are pH-dependent. For instance, at pH 7.0, cinnamon EO and its main component cinnamaldehyde resulted in higher acetate to propionate ratio whereas, at pH 5.5, the acetate to propionate ratio was lower with cinnamon EO and cinnamaldehyde.

There also appears to be an adaptive response to EO supplementation in the rumen at the bacterial and/or population level. Such a response is a major challenge to developing EO feed additives with long-lasting effects. The adaptive response is particularly evident when low levels of EO are supplemented. Cardozo et al. (2004) used cinnamon, garlic and anise oils (7.5 mg/kg DM or 0.22 mg/l) in continuous culture and observed changes in VFA profiles during the first 6 days of microbial adaptation but no effects thereafter. Similarly, Busquet et al. (2005b) studied effects of garlic oil on *in vitro* rumen microbial fermentation in a 24 h batch culture. Total VFA concentration decreased at a dose of 300 mg/l but, in a subsequent continuous culture study, there were no effects of garlic oil, at doses up to 312 mg/l, on total VFA concentration (Busquet et al., 2005a). These studies are compelling evidence that microbial populations are able to adapt to EO over time, which presents a challenge for commercial application of this feed additive technology.

5.3. Methane production

There is growing worldwide interest in reducing methane emissions from domestic ruminants. Methane is a potent greenhouse gas and its release into the atmosphere is directly linked with animal agriculture, particularly ruminant production. The antimicrobial activity of EO has prompted interest in whether these compounds could be used to inhibit methanogenesis in the rumen. The challenge is to identify EO that reduce methane produc-

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tion without a concomitant reduction in feed digestion. Evans and Martin (2000) observed that thymol (400 mg/l), 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. Kamra et al. (2005) investigated methanol and ethanol extracts of various spices, including fennel, clove, garlic, onion, and ginger for effects on methane production *in vitro*. Among the extracts tested, methanol extract of garlic was the most effective suppressant of methane, with a 64% reduction *in vitro* and no adverse effects on feed digestibility. Similarly, Busquet et al. (2005a) reported that garlic oil (312 mg/l) reduced acetate and increased propionate proportions in a manner consistent with decreased methane production *in vitro*, although methane was not directly measured. Evaluating the effects of garlic oil and four of its main components (diallyl sulfide, diallyl disulfide, allyl mercaptan, and allicin), Busquet et al. (2005b) observed, in batch culture, that garlic oil and diallyl disulfide (300 mg/l of ruminal fluid) reduced methane production by 74 and 69% respectively, without altering digestibility. Interestingly, monensin did not reduce methane production to the same extent as garlic oil or diallyl disulfide. Busquet et al. (2005b) suggested that garlic oil and diallyl disulfide did not exert their effects through the same mode of action as monensin, but rather that inhibition of methane production by these compounds was due to the direct inhibition of rumen methanogenic archaea. Patra et al. (2005) reported that ethanol and methanol extracts of cloves and the methanol extract of fennel also inhibited methane production *in vitro*, but digestibility of the feed was also reduced. The ground root from Rhubarb (*Rheum officinale*, 1.6 g/l) was reported to reduce methane production *in vitro* by 20% without affecting digestibility, whereas the bark from buckthorn (*Rhamnus frangula*, 1.6 g/l) had no effect on methane (García-González et al., 2005).

There may be potential to select EO compounds that reduce methane by selectively inhibiting protozoal numbers,which would be expected to decrease methane production because ruminal protozoa provide a habitat for methanogens that live on and within them. However, the antiprotozoal effects of EO have been inconsistent and variable among EO and EO active components (see Section 5.1.).

Few studies have evaluated effects of EO and their main components *in vivo* for effects on methane emissions, and no studies have assessed long-term effects of EO and their constituents on methane production. In one study, MEO was fed (1 g/day) to beef cattle consuming a high forage diet. Methane emissions were not affected, although feed digestibility decreased (Beauchemin and McGinn, 2006). Using the same commercial product in an *in vitro* study using pure cultures, McIntosh et al. (2003) reported that growth of the methanogen *Methanobrevibacter smithii* was inhibited, but only when the concentration of the product was 33 fold higher than that fed *in vivo*, as reported by Beauchemin and McGinn (2006), a feeding rate that is impractical due to potentially deleterious effects on diet digestibility. Using another high supplementation rate (*i.e.*, 20 g/kg of DM intake) of encapsulated horseradish, Mohammed et al. (2004) observed a 19% decrease in methane production in steers that was not accompanied by a reduction in protozoal numbers or feed digestibility. It seems clear that there is potential to select EO compounds that selectively reduce methane when used at levels that do not depress feed utilization, but further research is necessary to evaluate these compounds *in vivo*.

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6. Effects on ruminant performance

Few studies have been published on effects of EO or their constituents on milk production and composition of dairy cows. Benchaar et al. (2006b, 2007) observed no changes in DM intake, milk production, and milk components when dairy cows were fed 750 mg or 2 g of MEO daily. Similarly, supplementation of dairy cows with peppermint at 20 g/kg DM had no effect on milk yield and milk composition (Hosoda et al., 2005). More recently, Yang et al. (2006) observed that addition of garlic (*Allium sativa*, 5 g/day) and juniper berry (*Juniperus communis*, 2 g/day) oils to dairy cow diets had no effect on DM intake, milk production or milk composition. In these studies, the lack of effect of EO and their active components on milk performance was consistent with the absence of effects of these plant extracts on feed intake and ruminal fermentation.

Essential oils have an antibacterial activity against Gram-negative and Gram-positive bacteria (Helander et al., 1998). Several Gram-positive bacteria are involved in ruminal biohydrogenation of unsaturated dietary fatty acids (Harfoot and Hazlewood, 1988). Therefore, feeding EO could lower biohydrogenation of fatty acids by reducing the number, and the activity, of bacteria involved in the biohydrogenation of unsaturated fatty acids. Benchaar et al. (2007) reported no change in milk fatty acid profile when cows were supplemented daily with 750 mg of MEO. However, supplementing the same mixture at a higher concentration (*i.e.*, 2 g/day) increased the concentration of conjugated linoleic acid (CLA), a health-promoting fatty acid, in milk fat.

Data on effects of EO and their compounds on beef cattle performance are almost nonexistent. In one study, Benchaar et al. (2006a) evaluated growth performance of beef cattle fed a silage base diet supplemented with 2 or 4 g/day of a commercial mixture of EO compounds (Vertan®, IDENA, Sautron, France) consisting of thymol, eugenol, vanillin and limonene. Results showed that DM intake and average daily gain were not affected by the addition of this EO compounds mixture. However, the gain to DM intake ratio was affected quadratically with a dose of 2 g/day maximizing feed efficiency.

7. Control of pathogens

Although it has been shown that EO and its main constituents can inhibit several foodborne pathogens including *E. coli* O157:H7, *S. aureus*, *L. monocytogenes*, and *Salmonella* spp. (Burt and Reinders, 2003; Friedman et al., 2004; Penalver et al., 2005; Oussalah et al., 2007) the broad spectrum antimicrobial activity of these compounds may makes it difficult to use them to specifically target pathogens within the ruminant digestive tract. Those EO and EO compounds that exhibit the highest antimicrobial activity against ruminal bacteria (*e.g.*, carvacrol, oregano and thyme oils) are also generally the most potent against pathogens (Oussalah et al., 2007). In some instances, EO may also increase the sensitivity of bacteria to other antimicrobials (Rafii and Shahverdi, 2007; Santiesteban-Lopez et al., 2007) and it is possible that they may also enhance the sensitivity of pathogens within the digestive tract to other microbialcides. Recently, EO from peppermint was shown to exhibit activity against *Giardia* (Vidal et al., 2007) a protozoal parasite that is highly prevalent in cattle (Olson et al., 2004). It is possible that EO, or their active components, may also have activity against

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other parasites that reside in the intestine such as *Cryptosporidium*, coccidia or nematodes. Effects of EO on parasites in the lower digestive tract would be dependent on the ability of the antimicrobial components that they contain to remain active after passage through the rumen. To date, the extent to which EO escape from the rumen and flow to the lower digestive tract has not been examined.

8. Conclusions

Plant-derived EO may be a useful means to improve efficiency of nutrient utilization in ruminants and reduce the impact of their production on the environment. Most studies to date have been laboratory based (*i.e.*, *in vitro*) and of a short-term nature, but indicate that EO and their active components may favorably alter ruminal fermentation. At high doses, EO and their constituents may inhibit deamination of AA and reduce methane production in the rumen. However, long-term *in vitro* (*i.e.*, continuous cultures) and *in vivo* studies suggest that benefits associated with EO diminish over time due to shifts in microbial populations or adaptation of individual microbial species to EO. Consequently, it may be difficult to continue to realize benefits from EO throughout the feeding or lactation period. Several studies have shown that EO possess strong bactericidal activity against several food born pathogens. However, the extent to which microbial pathogens exhibit a similar adaptive response to EO is presently unknown. The range of EO and their components is complex in terms of nature and activity and variability in their composition may make it difficult to obtain consistent positive responses in ruminant production from these complex mixtures.

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