ESSENTIAL OILS AS RUMEN MODIFIERS IN DAIRY COWS: OPPORTUNITIES AND CHALLENGES

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INTRODUCTION

In livestock production, antibiotics (e.g. ionophores) at sub-therapeutic levels (2.5-125 mg/kg feed, i.e. 5-10 × lower than therapeutic levels) are commonly used to improve the efficiency of converting feeds to gain (milk and meat) and/or to prevent disease and metabolic disorders. Ionophores function by dissipitating ion gradients across cell membranes of susceptible bacteria (principally Gram-positive bacteria), which are selected against, resulting in beneficial changes to rumen fermentation (Callaway et al., 2003; Russell and Houlihan, 2003; Tedeschi et al., 2003). The proportion of propionate relative to acetate is increased, which is associated with a concomitant reduction in methane production, and the degradation of dietary protein in the rumen is reduced, both of which contribute to increased feed conversion efficiency. They also help reduce the risk of acidosis, ketosis, displaced abomasums, and bloat in cattle. Despite these positive effects on animal productivity and health, the use of feed antibiotics in animal nutrition has become increasingly controversial because of the emergence of multi-drug resistant bacteria that may pose a risk to human health. Consequently, in recent years, public pressure to restrict or even ban the widespread use of antibiotics in animal nutrition has increased. It is likely that such public pressure would eventually force producers to produce milk and meat with less or no antibiotic feed supplements in animal diets. For example, a recent report of the Pew Commission on Industrial Farm Animal Production in the United States (PCIFAP, 2008) recommended restricting the use of antimicrobials in food animal production (to reduce the risk of antimicrobial resistance to medically most-used antibiotics).

Plant natural products are potential alternatives to antibiotic feed additives and it is hoped they may permit a reduction in the use of synthetic antimicrobial drugs. Therefore, the interest in the medicinal properties of natural products (essential oils, herbs, spices, botanicals) as animal feed supplements with the potential of improving animal productivity and health and to mitigate the environmental impact of animal feeding operations has dramatically increased in recent years. A search in the CAB (Commonwealth Agricultural Bureau) International database (CABI, 2008; animal sciences subject area) with essential oil as a keyword returned 345 references between 1970 and 1990 and 3174 references since 1991. Research has been particularly intensive in Europe after the European Union ban in January 2006 of the use of antibiotics in animal feed (OJEU, 2003). This paper presents the current knowledge on the use of plant-derived essential oils as feed additives in ruminant nutrition, mainly dairy cow. Mechanisms of action, effects on rumen microbial fermentation (protein metabolism, volatile fatty acid production, methane production) and dairy cow performance are discussed.

DEFINITION

Essential oils are oily liquids composed of low molecular weight, hydrophobic (lipophilic) secondary metabolites extracted from plants. They have low boiling points, which renders them volatile and hence they are also referred to as volatile oils. Contrary to what their name might suggest, essentials oils are not "essential" in the nutritional sense of the word nor are they "oils" in the sense of being lipids. They are referred to as "essential" because they are the "essence" of plants – they are responsible for the fragrance and the flavour of plants.

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 essential oil can vary among different parts of the same plant (Dorman and Deans, 2000). For instance, essential oils obtained from the seeds of coriander (*Coriandrum sativum* L.) have a different composition from the essential oils of cilantro, which is obtained from the immature leaves of the same plant (Delaquis et al., 2002). Chemical differences among essential oils 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 example, Martínez et al. (2006) observed that the concentration of carvacrol, thymol, *p*-cymene and γ-terpinene in thyme essential oils varied widely depending on the species of the thyme plant (Figure 1).

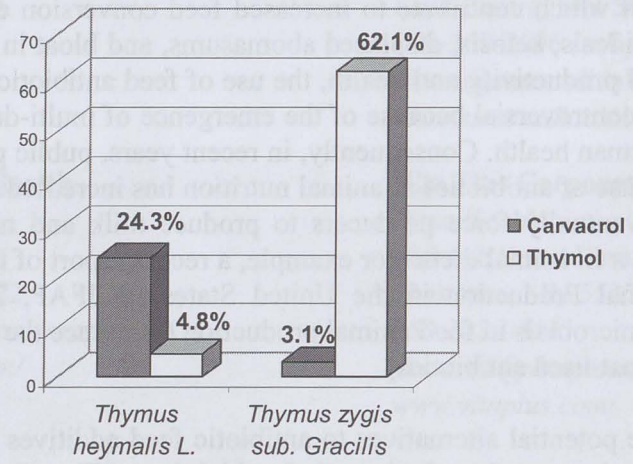

Figure 1: Concentration (%) of carvacrol and thymol of 2 species of thyme (adapted from Martínez et al., 2006).

CHEMISTRY

The building blocks of secondary metabolites are derived from primary metabolism, principally the processes of photosynthesis, glycolysis and the citric acid cycle. The most important building blocks are derived from acetyl-CoA, shikimic acid, mevalonic acid and deoxyxylulose (Figure 2).

The secondary metabolites that make up essential oils are normally either terpenes or phenylpropenes. When these compounds contain oxygen in their structure they are referred to as terpenoids and phenylpropanoids.

Terpenes and phenylpropenes are groups of secondary metabolites that are derived from common pathways and synthesised using common structural units. While compounds representative of both groups may be present in a plant's essential oil, normally compounds from one group dominate. For example, the major constituents of clove (*Eugenia caryophyllus*) essential oil are the phenylpropenes eugenol, eugenol acetate and ß-caryphyllene (e.g. 75-90%, 10-15% and 3% of typical composition, respectively), while the major constituents of oregano (*Oreganum vulgare*) essential oil are terpenes (i.e. carvacrol, thymol) .

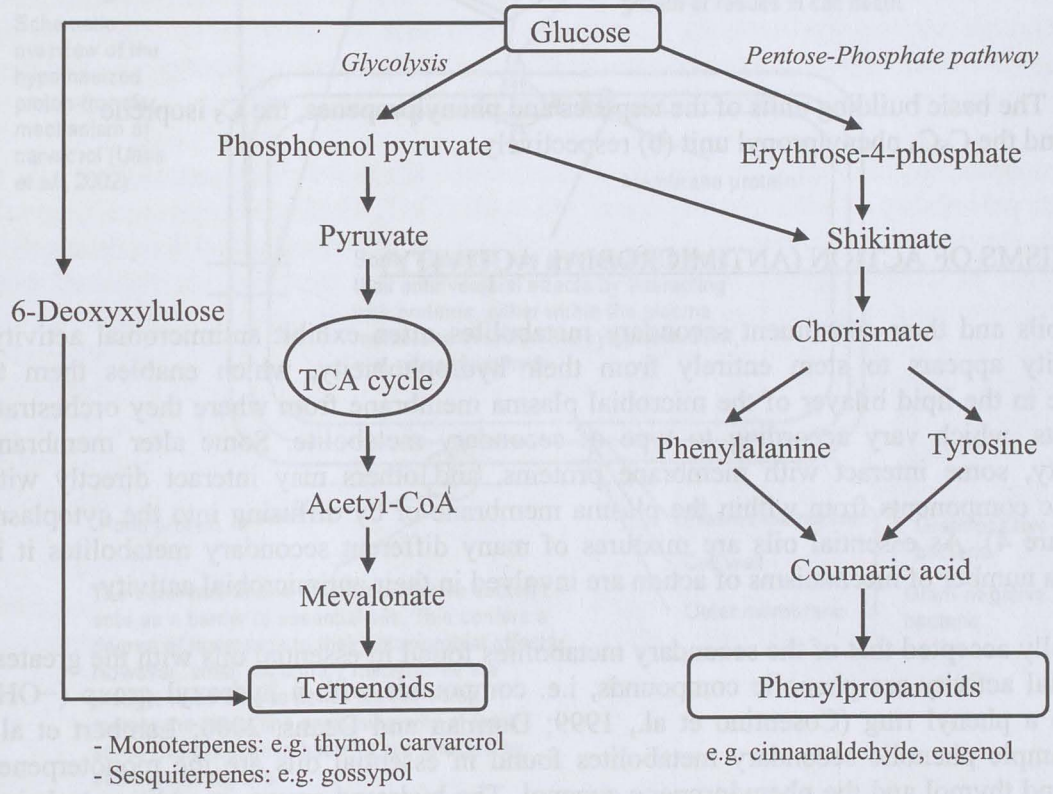

Figure 2. An overview of the pathways responsible for synthesising terpenes and phenlypropenes, the principle metabolites found in plant essential oils.

The basic building blocks of phenylpropenes are C_6C_3 phenylpropyl units (a 6-carbon aromatic ring with a 3-carbon chain attached to it; Figure 3), which are derived from the carbon skeletons of the aromatic amino acids phenylalanine and tyrosine. These amino acids, including tryptophan, are synthesised by the shikimate pathway, so named because shikimic acid is a key intermediate in the pathway.

The basic building blocks of the terpenes, the C_5 isoprene units (Figure 3) isopentenyl diphosphate and dimethylallyl diphosphate (hemiterpenes), are derived from the mevalonate and deoxyxylulose pathways.

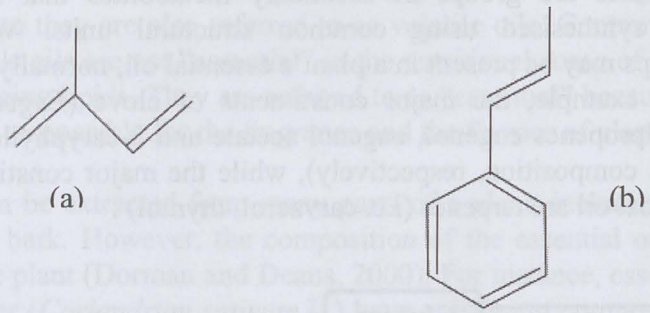

Figure 3. The basic building units of the terpenes and phenylpropenes, the C_5 isoprene unit (a) and the C_6C_3 phenylpropyl unit (b) respectively.

MECHANISMS OF ACTION (ANTIMICROBIAL ACTIVITY)

Essential oils and their constituent secondary metabolites often exhibit antimicrobial activity. This activity appears to stem entirely from their hydrophobicity, which enables them to accumulate in the lipid bilayer of the microbial plasma membrane from where they orchestrate their effects, which vary according to type of secondary metabolite. Some alter membrane permeability, some interact with membrane proteins, and others may interact directly with cytoplasmic components from within the plasma membrane or by diffusing into the cytoplasm itself (Figure 4). As essential oils are mixtures of many different secondary metabolites it is likely that a number of mechanisms of action are involved in their antimicrobial activity.

It is generally accepted that of the secondary metabolites found in essential oils with the greatest antimicrobial activity are phenolic compounds, i.e. compounds with a hydroxyl group (−OH) attached to a phenyl ring (Cosentino et al., 1999; Dorman and Deans, 2000; Lambert et al., 2001). Example phenolic secondary metabolites found in essential oils are the monoterpenes carvacrol and thymol and the phenylpropene eugenol. The hydroxyl group, in addition to being involved in the transport of ions across the plasma membrane (Ultee et al., 2002), is also thought to be involved in the inactivation of microbial enzymes (Burt, 2004).

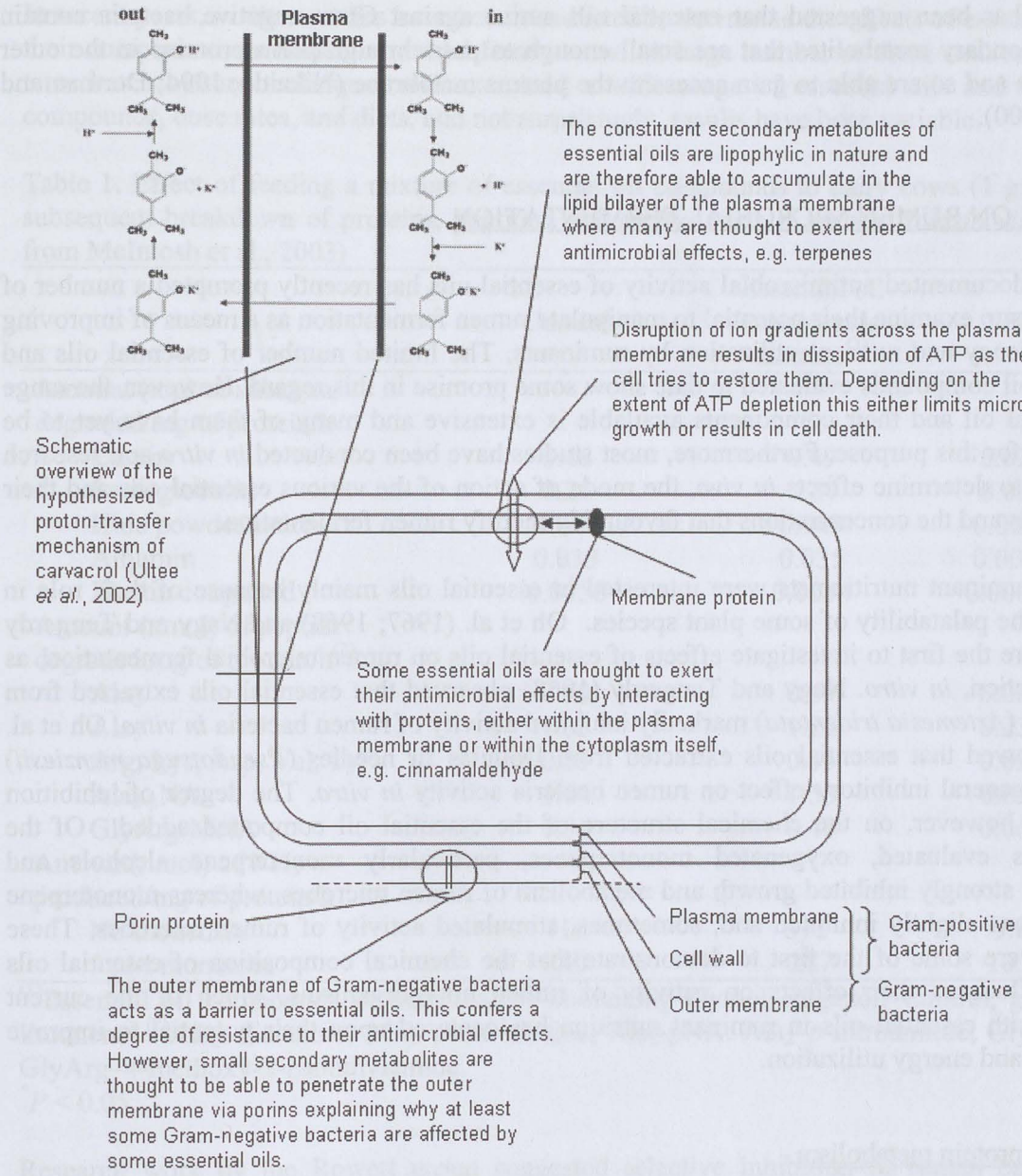

Figure 4. Schematic overview of some of the sites and mechanisms of antimicrobial activity of essential oils in the bacterial cell.

The antimicrobial activity of essential oils tends to be selective against the Gram-positive bacteria. For example, there is evidence to suggest that Gram-positive bacteria may be more sensitive to oregano essential oil and its constituent phenols than Gram-negative bacteria (Cosentino et al., 1999; Lambert et al., 2001) and Smith-Palmer et al. (1998) has reported similar observations for other essential oils. It has been hypothesized that the differences in sensitivity between these bacterial groups is due to the differences in the cell envelope, in that access of essential oils to the membrane is more restricted in Gram-negative bacteria (Helander et al.,

1998). It has been suggested that essential oils active against Gram-negative bacteria contain active secondary metabolites that are small enough to pass through porin proteins in the outer membrane and so are able to gain access to the plasma membrane (Nikaido, 1994; Dorman and Deans, 2000).

EFFECTS ON RUMEN MICROBIAL FERMENTATION

The well documented antimicrobial activity of essential oils has recently prompted a number of researchers to examine their potential to manipulate rumen fermentation as a means of improving feed efficiency and nutrient utilization by ruminants. The limited number of essential oils and essential oil compounds evaluated to date show some promise in this regard. However, the range of essential oil and their components 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 essential oils and their compounds and the concentrations that favourably modify rumen fermentation.

Initially, ruminant nutritionists were interested in essential oils 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 essential oils on rumen microbial fermentation, as gas production, *in vitro*. Nagy and Tengerdy (1968) observed that essential oils extracted from Sagebrush (*Artemesia tridentata*) markedly inhibited activity of rumen bacteria *in vitro*. Oh et al. (1967) showed that essential oils extracted from Douglas fir needles (*Pseudotsuga menziesii*) exerted a general inhibitory effect on rumen bacteria activity *in vitro*. The degree of inhibition depended, however, on the chemical structure of the essential oil 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 essential oils greatly influences their effects on activity of rumen microorganisms. Much of the current research with essential oils in ruminant nutrition has centered upon their potential to improve ruminal N and energy utilization.

Effects on protein metabolism

Symbiosis between ruminants and their microflora instills 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.

In recent years, *in vitro* studies have been conducted to determine the effects of essential oils and their components on rumen microbial fermentation. A large number of these studies used short-term batch culture techniques and examined a wide range of essential oils and essential oil compounds, dose rates, and diets, and not surprisingly, results have been variable.

Table 1. 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	Essential oil compounds mixture[1]	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[2]			
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
$Ala_{2\text{-}p}NA$	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
5 μM monensin	280	287	10.9

[1]Essential oil compounds mixture (MEO) containing thymol, eugenol, vanillin, guaiacol and limonene. [2]Ala_2: dialanine; Ala_5: penta-alanine; Ala_2-pNA: Ala_2–*p*-nitroanilide; GlyArg-MNA: GlyArg–4-methoxy-2-naphthylamide.
*$P < 0.05$.

Research work by the Rowett group suggested selective inhibition of rumen bacteria by a specific mixture of essential oil compounds (MEO) that included thymol, eugenol, vanillin, and limonene (McIntosh et al., 2003). Using 48 h *in vitro* batch culture incubations, McIntosh et al. (2003) observed reduced (−9%) deaminative activities of rumen fluid collected from dairy cows fed a silage-based diet supplemented with 1 g/day of MEO (Table 1). The inhibitory effect of this particular mixture on deaminative activity was later confirmed *in vitro* (24 h batch cultures) by Newbold et al. (2004) who reported a decrease (−25%) in deaminative activities of rumen fluid collected from sheep fed 110 mg/day of MEO. However, in the later study *in vivo* rumen concentration of ammonia N was not affected by the addition of the specific commercial mixture. In further investigations, McIntosh et al. (2003) observed that MEO inhibited the growth of some hyper-ammonia producing (HAP) bacteria such as *Clostridium sticklandii* and *Peptostreptococcus anaerobius* while the growth of other HAP bacteria (e.g. *Clostridium*

aminophilum) were not affected (Table 2). Castillejos et al. (2005, 2007) did not find MEO effective in modifying ruminal N metabolism (concentrations of ammonia N, large peptide N, small peptide plus amino acid N, bacterial and dietary N flows, degradation of N, and efficiency of microbial protein synthesis) when added at the concentrations of 1.5, 5, 50, and 500 mg/l in a continuous culture fermenter maintained at constant pH (6.4 ± 0.05). The lack of effectiveness of MEO in those studies may be attributed to the dosage rates used, which may not have been high enough to affect microbial populations. In fact, McIntosh et al. (2003) reported that a minimal concentration of 40 mg/l of MEO is required in order to inhibit the growth of predominant rumen bacterial species, including HAP bacteria (e.g. *Clostridium sticklandii* and *Peptostreptococcus anaerobius*). This concentration is higher than that likely to be achieved *in vivo* (Hart et al., 2008). In fact, results from *in vivo* studies (Benchaar et al., 2006, 2007a) showed that feeding MEO (0.75 and 2 g/day) to dairy cows had no effects on rumen ammonia N concentration, retention and digestibility of N. Such feeding levels of 0.75 and 2 g/day would correspond to a maximum rumen concentration of 7.5 and 20 mg/l, respectively, assuming an average rumen volume of 100 L for an adult dairy cow. These rumen concentrations are indeed much lower than the reported concentration of 40 mg/l required for MEO to alter substantially N metabolism in the rumen (McIntosh et al., 2003).

Table 2: Effect of essential oils on growth of pure cultures of HAP bacteria after direct inoculation or after adaptation (adapted from McIntosh et al., 2003)

	IC_{50} of MEO (ppm)*	
Strain	Direct inoculation	Adaptation (48 h)
Clostridium sticklandii 12662	36.0	35.0
Peptostreptococcus anaerobius 27337	42.5	52.0
Prevotella ruminicola 23	33.8	94.3
Prevotella bryantii B14	54.0	127.5
Clostridium aminophilum 49906	94.2	262.5

* IC_{50} is the concentration of essential oils that led to a 50% decrease in cell density at 24 h of incubation.
MOE = Essential oil compounds mixture containing thymol, eugenol, vanillin, guaiacol and limonene.

The effects of essential oils on rumen protein metabolism have also been assessed using the *in situ* bag technique and the results were variable among studies depending on the protein source tested, the composition of the diet and the dose of the product fed to animals. Molero et al. (2004) observed small reductions in the effective rumen degradabilities of protein in lupin seeds (−3%), green peas (−6%), and sunflower meal (−5%) when growing heifers were supplemented for 10 days with 700 mg/day of MEO in a high-concentrate diet. When MEO was added to a low-concentrate diet, only the rumen degradability of green peas was slightly reduced (−2%). Based on the results of Molero et al. (2004), Hart et al. (2008) speculated that the effect of MEO on rumen degradation of protein seems to be selective with the effects being more pronounced with the rapidly degradable protein sources than with the more resistant substrates. Nevertheless, the observed reductions were too small to have any likely nutritional impact on rumen protein metabolism in the animal.

There are some suggestions that essential oils may be effective only after a prolonged period of exposure/adaptation of the rumen microorganisms to the active compounds. Castillejos et al. (2007) speculated that an adaptation time of 28 days is necessary to observe an effect of MEO on ruminal N metabolism. However, in the study by Molero et al. (2004), when the adaptation period was extended to 28 days (*versus* 10 days), the addition of 700 mg/day of MEO to a high-forage diet did not modify the effective rumen degradability of soybean meal N, although MEO decreased the soluble and increased the potentially degradable N fractions. In another *in situ* study, Newbold et al. (2004) examined the effects of MEO on rumen degradation of soybean meal N incubated at different time intervals (0, 2, 4, 6, 8, 16, 24, and 48) in the rumen of adult sheep fed for 42 days a high-forage diet supplemented with 110 mg/day of MEO. Results showed that rumen degradation of soybean meal N was only reduced (−18%) at 2 h incubation. However, it is unlikely that this change would have had an impact on the overall effective rumen degradability of soybean meal N. More recently, Benchaar et al. (2006, 2007a) reported no changes in protein degradability of soybean meal incubated in the rumen of lactating dairy cows fed MEO at 0.75 or 2 g/day. Collectively, results from continuous culture studies (Castillejos et al., 2005, 2007), *in situ* (Molero et al., 2004; Newbold et al., 2004; Benchaar et al., 2006, 2007a), and *in vivo* (Benchaar et al., 2006, 2007a) studies failed to confirm the positive effects of MEO additive on rumen N metabolism reported in short-term batch culture incubation studies (McIntosh et al., 2003; Newbold et al., 2004). This discrepancy between studies using different experimental approaches is a clear illustration that short-term *in vitro* studies have limitations and that the ultimate value of essential oils for altering rumen microbial fermentation must be assessed *in vivo*.

The range of essential oils available is extensive and many of these substances have yet to be examined for their antimicrobial effects against rumen microbes. In addition to studies investigating the effects of commercial mixtures of essential oils, other studies evaluated in depth the potential of single, naturally occurring essential oils and their main components to modulate rumen microbial fermentation. Most of these studies are laboratory based (batch culture or continuous culture systems) and originate from the group of the University of Barcelona (Spain). The effects on rumen N metabolism were variable depending on the essential oil or the essential oil compound being tested and the dose used. Cardozo et al. (2004) reported that the addition (0.22 mg/l) of cinnamon bark essential oil (*Cinnamonum cassia*; 59% cinnamaldehyde) in a continuous culture fermenter maintained at constant pH did not affect ammonia N concentration, but increased the concentration of peptide N and numerically decreased that of amino acid N, suggesting that peptidolysis was inhibited. In subsequent studies from the same laboratory, no changes were observed in N metabolism (i.e. concentrations of ammonia N, large peptide N, and small peptide plus amino acid N) when cinnamaldehyde was supplied at higher concentrations (i.e. 2.2, 31.2 and 312 mg/l of culture fluid) in continuous culture systems (Busquet et al., 2005a; 2005b). Using *in vitro* batch culture incubations (24 h), Busquet et al. (2006) observed that high doses of cinnamon oil (3000 mg/l) and cinnamaldehyde (300 and 3000 mg/l) strongly inhibited ammonia N concentration, but the effects were non-existent at low doses (i.e. 3 and 30 mg/l). However, the decreased rumen ammonia N concentration was associated with a reduction in total VFA concentration, suggesting a reduction in overall fermentation of the diet.

Few studies have investigated *in vivo* the effects of cinnamon oil and cinnamaldehyde on rumen microbial fermentation. Chaves et al. (2008a) observed no changes in rumen ammonia N concentration in lambs fed barley- or corn- based diets supplemented with cinnamaldehyde (200 mg/kg of dry matter intake). More recently, Benchaar et al. (2008) reported that supplementing lactating dairy cows with 1 g/day of cinnamaldehyde (i.e. 43 mg/kg of dry matter intake) had no effects on rumen concentration of ammonia N and *in situ* degradation of soybean meal N.

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). A number of *in vitro* studies have examined the effects of phenolic compounds (eugenol, carvacrol, and thymol) or essential oils with high concentrations of phenolic compounds on ruminal N metabolism. Reported effects have been variable among studies, apparently related to the dosage level and the *in vitro* technique used (batch vs. continuous culture). For instance, Busquet et al. (2005c) reported that addition of clove bud essential oil (*Syzygium aromaticum*; containing 85% of eugenol) at 2.2 mg/l to a continuous culture fermenter markedly decreased (−80%) large peptide N concentration, but had no effect on ammonia N concentration, suggesting that clove bud essential oil inhibited the peptidolytic activity of rumen bacteria. However, the addition of eugenol (main component of clove bud essential oil) at the same concentration had no effect on N metabolism, suggesting that the anti-peptidolytic activity of clove bud essential oil is not due to its main component, eugenol, but results from unidentified compounds within the essential oil fraction. Using a 24 h *in vitro* batch culture, Busquet et al. (2006) reported that, when supplied at the concentration of 3000 mg/l, both oregano essential oil and its major constituent carvacrol reduced the concentration of ammonia N, indicating that carvacrol is responsible for the majority of the antimicrobial activity in oregano essential oil. Controversial results for cinnamon leaf essential oil (containing 76% of eugenol) were reported by Fraser et al. (2007). The addition of cinnamon leaf essential oil (500 mg/l) in the Rusitec (Rumen Simulation Technique) fermenter decreased ammonia N concentration and molar proportions of branched-chain volatile fatty acids (end-products fermentation of branched amino acid catabolism in the rumen), whereas no effects were observed in the dual-flow system. More recently Benchaar et al. (2008) and Chaves et al. (2008b) reported that eugenol (800 mg/l) and cinnamon leaf essential oil (250 mg/l) had no effects on deaminative activity (expressed as μg ammonia N/mg bacterial N/min) of rumen bacteria and ammonia N concentration *in vitro*.

Thymol has been extensively investigated for its antimicrobial properties against different types of microorganisms, including rumen microbes. An early report by Borchers (1965) showed that the addition of thymol (1000 g/l) to rumen fluid containing casein resulted in an accumulation of amino acid N and a decrease in ammonia N, suggesting inhibition of amino acid deamination by rumen bacteria. Castillejos et al. (2006; 2008) conducted a series of batch culture and continuous culture studies to assess the potential of thyme oil (*Thymus vulgaris*) and its main constituent thymol, to favourably alter rumen microbial fermentation. In general, at low to moderate doses (5 and 50 mg/l) thymol had no effects on ruminal N metabolism. At high doses of thymol (500 and 5000 mg/l) results on N metabolism were inconsistent depending on the *in vitro* system used. At these doses, thymol decreased ammonia N and branched-chain volatile fatty acids concentrations in 24 h batch cultures, which is consistent with the inhibition of the deamination process. However, when added at the concentration of 500 mg/l in a continuous culture system,

thymol increased the concentration of large peptide N and small peptide plus amino acid N, but had no effect on ammonia N concentration. The accumulation of large peptides N and small peptide plus amino acid N is an indication that both proteolysis and peptidolysis processes were stimulated by thymol. At the concentrations of 5, 50, and 500 mg/l, thyme oil reduced ammonia N concentration but had no effect on that of branched-chain volatile fatty acids in 24 h *in vitro* batch culture fermentation (Castillejos et al., 2008). Hristov et al. (2008) examined the effects of 40 essential oils (at 10 and 100 mg/l final medium concentration) on rumen fermentation in short-term (4 h) *in vitro* batch culture incubations. Of the 40 essential oils evaluated, very few had statistically significant effects on ammonia N concentration. However, the observed effects were subtle and the authors concluded that it was unlikely that these moderate *in vitro* effects would correspond to any substantive impact on rumen N metabolism *in vivo*.

Additive, antagonistic, and synergistic effects have been observed between components of essential oils (Burt, 2004). This suggests that combinations of essential oils of different composition or specific combinations of essential oil secondary metabolites may result in additive and/or synergetic effects that may enhance efficiency of rumen microbial fermentation and nutrient utilization in ruminants. For example, Cardozo et al. (2006) evaluated the effects of feeding a mixture of cinnamaldehyde and eugenol in beef cattle fed a diet consisting of 90% of concentrate and 10% of barley straw. The combination of these two essential oil compounds at two feeding rates (180 mg/day of cinnamaldehyde + 90 mg/day of eugenol; 600 mg/day of cinnamaldehyde + 300 mg/day of eugenol) affected N metabolism in the rumen by increasing the concentration of small peptide plus amino acid N (+10%) and decreasing ammonia N concentration (−9%), suggesting that deamination was inhibited.

Effects on volatile fatty acid production

It may be energetically favourable to the animal if essential oils increase volatile fatty acid (VFA) production, alter VFA profile such that proportionally more propionate and less acetate are produced, and decrease methane production during rumen fermentation.

The effects of essential oils and their constituents on volatile fatty acid production have been inconsistent among studies ranging from no change to increased or decreased total rumen VFA concentration. Castillejos et al. (2005) observed that the addition of 1.5 mg/l of MEO supplement increased total VFA concentration without affecting proportions of individual VFA in a continuous culture system maintained at constant pH. However, the increase in total VFA concentration was not consistent with the lack of effects of MEO additive on organic matter digestibility. In a later continuous culture study by the same group (Castillejos et al., 2007), supplementation with MEO at 5 mg/l increased total VFA concentration and shifted VFA pattern towards more acetate and less propionate, although again there was no concomitant increase in organic matter digestibility. When MEO was added at higher concentrations (50 and 500 mg/l) in the same study, there were no effects on total VFA concentration, VFA pattern and digestibility of nutrients. The authors offered no explanation for this lack of effects. Castillejos et al. (2007) suggested that rumen microbes need to be exposed to the MEO for at least 6 days to observe changes in VFA. However, in their study, total VFA concentration was not affected both *in vivo* and *in vitro* in rumen fluid collected from sheep adapted for 4 weeks to MEO.

Other *in vivo* studies (Newbold et al., 2004; Beauchemin and McGinn, 2006; Benchaar et al., 2006) reported no changes in total VFA concentration and VFA proportions when MEO was fed to sheep (110 mg/day), beef cattle (1 g/day), and lactating dairy cows (2 g/day). Benchaar et al. (2007a) observed that rumen total VFA concentration tended to increase (+5%) in dairy cows fed an alfalfa silage-based diet supplemented with MEO (750 mg/day), but tended to decrease (−10%) when the diet contained corn silage. Although the observed changes were small, such results may suggest that effects of MEO on total VFA concentration may be diet depend.

The effects of pure essential oils and their main components on total VFA concentration have been shown to be dose dependent. Busquet et al. (2006) screened several essential oils (anise, cade, capsicum, cinnamon, clove, bud, dill, garlic, ginger, oregano, and tea tree) and essential oil compounds (anethol, benzyl salicylate, carvacrol, carvone, cinnamaldehyde, and eugenol) for rumen effects when supplied at 3, 30, 300, and 3000 mg/l in 24 h batch culture fermentations. At low (3 mg/l) and moderate (30 mg/l) doses, none of the essential oils or essential oil compounds affected total VFA concentration. At high doses (300 and 3000 mg/l) most of treatments decreased total VFA concentration. Castillejos et al. (2006) also observed that high doses (500 to 5000 mg/l of culture fluid) of some essential oil compounds (eugenol, guaiacol, limonene, thymol, and vanillin) strongly decreased total VFA concentration in 24 h batch cultures of rumen fluid. A reduction in total VFA production may be a reflection of reduced diet fermentability and would generally be viewed as nutritionally unfavourable because VFA are the main source of metabolizable energy to ruminants.

A number of *in vitro* studies have shown that some essential oils and essential oil compounds produce desirable changes in rumen fermentation by shifting VFA profile towards more propionate and less acetate. For example, in a continuous culture study, Busquet et al. (2005b) reported that at the doses of 31.2 and 312 mg/l, cinnamaldehyde decreased the proportion of acetate and increased the proportions of propionate and butyrate. Other *in vitro* studies reported that garlic oil also reduced acetate proportion and increased propionate and butyrate proportions (Busquet et al., 2005b; 2006). High butyrate concentration as a result of supplementation with cinnamaldehyde and garlic oil may indicate that these secondary metabolites act differently from monensin, but similarly to other methane inhibitors.

While previous studies have shown that the use of some essential oils may result in beneficial effects on VFA profile, other studies, however, revealed that the use of some essential oils and essential oil constituents results in undesirable changes in the proportions of individual VFA. For instance, in a batch culture study Castillejos et al. (2006) observed that at 500 mg/l, thymol decreased total VFA concentration, increased acetate proportion and reduced that of propionate. Benchaar et al. (2007b) reported that carvacrol (400 mg/l) and eugenol (800 mg/l) reduced the molar proportion of propionate without affecting total VFA concentration in batch culture incubations. It is possible that the effect of essential oils on VFA profile may be diet and pH dependent as shown in an *in vitro* batch culture study by Cardozo et al. (2005). For example, at pH 7.0 cinnamaldehyde and capsicum increased the acetate to propionate ratio, while at pH 5.5 the acetate to propionate ratio was lower with cinnamaldehyde and capsicum.

Overall, supplementation with essential oils and or their main constituents caused either a decrease or no change in total VFA concentration in most studies. In some studies essential oils

alter favourably VFA pattern, whereas in others essential oils produce undesirable changes in the proportions of individual VFA. The challenge is to identify the dose rates for various essential oils or essential oil compounds that favourably alter aspects of rumen metabolism without reducing total VFA concentrations.

Microbial populations exhibit a remarkable capacity to adapt to and/or degrade a wide variety of plant secondary metabolites such as saponins and tannins (Newbold et al., 1997; Makkar et al., 1995; Makkar, 2003). Similarly with essential oils, there appears to be adaptation of rumen microbial populations, particularly at low dosage rates *in vitro*. Indeed, rumen microbes were able to adapt to essential oils when these secondary metabolites were administered at low doses (Cardozo et al., 2004: 0.22 mg/l; Busquet et al., 2005a: 2.2 mg/l), but at higher doses (Busquet et al., 2005b: 300 mg/l; Fraser et al., 2007: 500 mg/l), the effect of essential oils appears to be sustained over time (e.g. 7 to 9 days of continuous culture fermentation). However, such concentrations ($\geq$ 300 mg/l) are higher than likely to occur *in vivo* and would correspond to impractical feeding rates that if applied, would adversely affect the efficiency of rumen microbial fermentation and animal performance. Results from these studies provide evidence that under practical feeding conditions (i.e. normal feeding rates), microbial populations are able to adapt to essential oil over time, which represents a challenge for commercial application of this feed additive technology.

Effects on methane production

There is growing worldwide interest in reducing methane emissions from domestic ruminants. Methane formation represents a net loss of energy to the host animal. Energy lost as methane from cattle ranges from 2 to 12% of gross energy intake (Johnson and Johnson, 1995). Methane is also a potent greenhouse gas and its release into the atmosphere is directly linked with animal agriculture, particularly ruminant production. Mitigating methane emissions from ruminants will have short-term economic (i.e. improved feed efficiency) and long-term environmental (i.e. decreasing agriculture's contribution to greenhouse gas emissions) benefits.The antimicrobial activity of essential oils has prompted interest in whether these compounds could be used to inhibit methanogenesis in the rumen.

A number of *in vitro* studies have evaluated the potential of essential oils to inhibit rumen methanogenesis. The reported effects varied with the type and the dose of the essential oil used. Evans and Martin (2000) observed no effects on methane concentration when thymol was used at 50, 100, and 200 mg/l of culture fluid in 24 h incubations of mixed rumen bacteria. However, at high concentration (400 mg/l), thymol strongly decreased methane concentration, but acetate and propionate concentrations were also reduced. Busquet et al. (2005b) studied the effects of high concentration (300 mg/l) of garlic essential oil and four of its main components (diallyl sulfide, diallyl disulfide, allyl mercaptan, and allicin) in batch culture fermentation (17 h). Garlic oil and diallyl disulfide drastically reduced methane production (−74 and −69% respectively), but diet digestibility and total VFA concentration were also depressed. In the same study, the inhibitory effect of monensin on methane was less pronounced (−42%) than garlic essential oil and diallyl sulphate. Based on these observations, Busquet et al. (2005b) suggested that contrarily to monensin, which specifically inhibits rumen Gram-positive bacteria, the antimethanogenic effect of garlic essential oil and its main components was the result of a direct inhibition of Archaea

microorganisms in the rumen. In an *in vitro* short-term incubation study (6 h), Chaves et al. (2008b) observed that cinnamon leaf oil (250 mg/l), garlic oil (100 and 250 mg/l), juniper berry oil (20 mg/l), and *p*-cymene (20 mg/l) reduced the methanogenic activity of ruminal bacteria (expressed as μmol of methane/g bacterial N/min) and methane concentration in the fermentation gases, without altering total VFA. Tatsuoka et al. (2008) investigated the effects of essential oil (cineol, eucalyptus, menthol, peppermint, thyme, and wasabi) cyclodextrin (CD, α or β) complexes on *in vitro* short-term (6 h) rumen fermentation. Eucalyptus-αCD (10 and 20 mg equivalent oil/60 mL of culture fluid) reduced methane production, increased total VFA concentration and the molar proportion of propionate. Wasabi oil (as α- or βCD, equivalent to 10 mg oil/60 mL of culture fluid) drastically reduced methane production and increased concentration of propionate. The other essential oil-CD showed no significant effect on reducing methane production.

Only few studies have examined *in vivo* the effect of essential oils and their main components on enteric methane emission by ruminants. Mohammed et al. (2004) observed that at high levels of feed incorporation (20 g/kg of dietary dry matter), encapsulated (α- cyclodextrin) horseradish oil decreased (−19%) methane production in steers without affecting diet digestibility. The reduced methane production was accompanied by a shift in VFA profile towards proportionally more propionate and less acetate and a decrease in the total numbers of methanogens. In another *in vivo* study, Beauchemin and McGinn (2006) observed no change in methane production, although feed digestibility decreased in beef cattle supplemented with MEO (1 g/day) in a high-forage diet. McIntosh et al. (2003) observed that the inhibition of the growth of the methanogen *Methanobrevibacter smithii* occurred only when the concentration of MEO exceeded 1000 mg/l. This level was 33-times higher than that fed in the *in vivo* study (33 mg/l of ruminal fluid) reported by Beauchemin and McGinn (2006), a feeding rate that is not practical, due to potentially adverse effects on efficiency of rumen fermentation and diet digestibility.

Based on findings from these studies, it appears that some essential oils and essential oil compounds have the potential to reduce enteric methane emission in ruminants. However, the challenge is to identify essential oils and components that selectively inhibit rumen methanogenesis without depressing feed digestion.

Effects on rumen ciliate protozoa

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. Moreover, improved efficiency of N metabolism in the rumen could reduce N losses in feces and urine. Reducing protozoal numbers often lowers rumen methanogenesis because ciliate protozoa have a symbiotic relationship with methanogenic bacteria. About 25% of rumen methanogens live in association with protozoa (Newbold et al., 1995). Thus, control of rumen protozoa population may offer a way to improve N and energy utilisation in ruminants. Due to the lack of a suitable defaunating agent, and spontaneous refaunation, defaunation has not been practical in commercial ruminant production systems.

Table 3: Effects of essential oils on protozoa counts in ruminal fluid of dairy cows

References	Product	Dose (mg/d)	Effect
Benchaar et al. (2006)	MEO	750	NS
Benchaar et al. (2007)	MEO	2000	NS
Yang et al. (2007)	Garlic	5000	NS
Yang et al. (2007)	Juniper berry	2000	NS
Benchaar et al. (2008)	Cinnamaldehyde	1000	NS
Benchaar et al. (2009)	Eugenol	1000	NS

MEO = thymol, eugenol, vanillin and limonene.
NS = non significant.

Plant extracts, such as condensed tannins and steroidal saponins, have been extensively investigated for their inhibitory effects on ciliate ruminal protozoa (Wallace, 1994; Wang et al., 1996; Wang et al., 2000; Min et al., 2002). However, few studies have examined the effects of essential oils on rumen ciliate protozoal populations (Table 3). Ando et al. (2003) reported a strong decrease in the total numbers of protozoa (−50%), as well as the numbers of certain protozoal species including *Entodinum* (−58%), *Isotrica* (−30%) and *Diplodium* (−70%), in rumen fluid from steers supplemented with 200 g/day (i.e. 54 g/kg of dry matter intake) of sun-dried peppermint (*Menthaxpiperita L.*). Mohammed et al. (2004) observed no modification in the number of rumen cilate protozoa when cyclodextrin encapsulated horseradish was included as a supplement at high doses both *in vitro* (0.17 to 1.7 g/l of culture fluid) and *in vivo* (20 g/kg of dietary dry matter). In a long-term (16 days incubation) continuous culture fermentation study, Fraser et al. (2007) observed that at 500 mg/l of culture fluid, cinnamon leaf oil (76% eugenol) reduced protozoa numbers in the RUSITEC and in a dual-flow fermenter. Cardozo et al. (2006) reported no change in entodiniomorphs and an increase in holotrichs numbers in beef heifers fed a high-concentrate based diet supplemented with a mixture of cinnamaldehyde (24 mg/kg of dry matter intake) and eugenol (12 mg/kg of dry matter intake). However, no effects were observed when the mixture contained higher concentrations of cinnalmadehyde (77 mg/kg of dry matter intake) and eugenol (38 mg/kg of dry matter intake). In the same study, feeding anise oil at 2 g/day (i.e. 250 mg/kg of dry matter intake) decreased the counts of entodiniomorphs and holotrichs whereas feeding capsicum oil at 1 g/day (i.e. 120 mg /kg of dry matter intake) had no effects. More recently, Benchaar et al. (2008) reported that supplementing dairy cow diets with 1 g/day of cinnamaldehyde (i.e. 43 mg/kg of dry matter intake) had no effect on total numbers of protozoa as well as the numbers of *Dasytricha*, *Diplodinium*, *Entodinium*, and *Polyplastron*. However, numbers of *Isotricha* increased. Newbold et al. (2004) and Benchaar et al. (2007a) reported that rumen protozoa counts were not affected when sheep and dairy cows were fed 110 and 750 mg/day of MEO, respectively. Rasmussen et al. (2005) reported that at the concentration of 100 mg/l, rosemary (*Rosmarinus officinalis*) essential oil had no effect on protozoa viability whereas at 10000 and 40000 mg/l, the essential oil greatly decreased (−90%) protozoal viability. However, again, these levels are very high and impractical in terms of feeding due to potentially negative effects on the efficiency of fermentation in the rumen. These studies suggest that essential oils and their main constituents have no marked effects on rumen ciliate protozoa when

supplied at low or moderate concentrations and it seems that high concentrations are required to exert an effect.

The effects of essential oils and their main components on rumen microbial fermentation are inconsistent and dose-dependent. The effects on N metabolism in the rumen are small and variable, and in most cases occur only after a strong inhibition of overall fermentation as evidenced by a reduction in total VFA concentration. The effects on total and individual VFA production are inconclusive. Only few studies examined the effect of essential oils and their compounds on rumen methanogens and methane production. In most cases, it appears that essential oils have a potential (through direct inhibition of methanogenic bacteria or inhibition of rumen protozoa) to inhibit methane production in the rumen, although this effect was accompanied by a reduction in diet digestibility.

EFFECTS ON PERFORMANCE

While a large number of *in vitro* studies have been published on the effects of essentials oils and their main constituents on rumen microbial fermentation, only few studies have been carried out to determine their effects on ruminant performance (Table 4).

Table 4: Effects of essential oils on dairy cow performance

References	Product	Dose (mg/d)	DMI (kg/d)	Milk (kg/d)	Fat (%)	Protein (%)
Benchaar et al. (2006)	MEO	750	NS	NS	NS	NS
Benchaar et al. (2007)	MEO	2000	NS	NS	NS	NS
Yang et al. (2007)	GAR	5000	NS	NS	NS	NS
Yang et al. (2007)	JUN	2000	NS	NS	NS	NS
Benchaar et al. (2008)	CDH	1000	NS	NS	NS	NS
Kung et al. (2008)	MEO	600	↑	↑	↑	NS
Benchaar et al. (2009)	EUG	1000	NS	NS	NS	NS

MEO = thymol, eugenol, vanillin and limonene
GAR = garlic; CDH = cinnamaldehyde; JUN = juniperberry; EUG = eugenol.
DMI = dry matter intake.
NS = non significant.

Several commercial products are currently available on the market that claim to improve feed efficiency and performance (milk and gain) when included in ruminant diets. Among these products, the MEO supplement is perhaps the most investigated. Benchaar et al. (2006; 2007a) observed no changes in dry matter intake, milk production and milk constituents when MEO additive was fed to dairy cows at the rates of 0.75 (i.e. 43 mg/kg of dry matter intake) or 2 g (i.e. 87 mg/kg of dry matter intake) daily. It is possible that the effect of MEO on milk composition is diet dependent. Yield of fat corrected milk (4% FCM) was not affected when MEO was added to alfalfa silage- or grass silage- based diets (Benchaar et al., 2006; Benchaar et al., 2007a), but it was depressed when the diet contained corn silage (Benchaar et al., 2006). Kung et al. (2008) reported higher dry matter intakes for lactating cows fed 1 g/day (i.e. 42 mg/kg of dietary dry

matter) of MEO. However, milk production was not significantly affected, although it was numerically increased in cows fed the MEO-supplemented diet (1.9 ± 0.9 kg/day; $P = 0.16$). In the later study by Kung et al. (2008), cows supplemented with MEO produced more fat-corrected milk than did the cows fed the control diet. Yang et al. (2007) observed that feeding garlic oil at 5 g/day (i.e. 245 mg/kg of dry matter intake) and juniper berry oil at 2 g/day (i.e. 98 mg/kg of dry matter intake) to lactating dairy cows had no effect on intake, milk production and milk composition. More recently, Benchaar et al. (2008) reported no changes in dry matter intake, milk production and milk components of dairy cows fed cinnamaldehyde at the dose of 1g/day (i.e. 43 mg/kg of dry matter intake).

Recently, plant secondary metabolites such as essential oils have been suggested as potential means to manipulate bacterial populations involved in rumen biohydrogenation in order to improve the fatty acid composition of ruminant-derived food products such as milk and meat. For instance, Durmic et al. (2008) observed that ethanolic extracts and essential oils from some Australian plants selectively inhibited the growth of pure cultures of some bacteria (e.g. *Clostridium proteoclasticum*) involved in rumen biohydrogenation and that some inhibited the saturation of linoleic acid, conjugated linoleic acid and vaccenic acid in batch culture incubations. This observation demonstrated the potential of plant extracts to increase output of conjugated linoleic and vaccenic acids from the rumen, and to enhance the concentrations of these potentially beneficial unsaturated fatty acids in ruminant-derived food products.

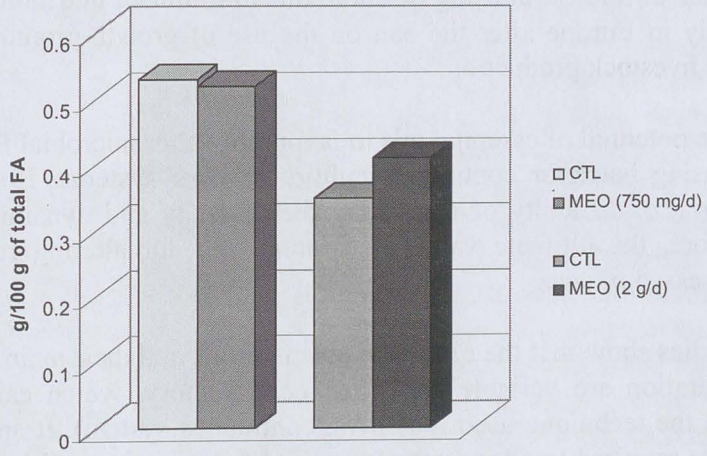

Figure 5: Effects of essential oils (MEO; CTL = Control) on the concentration of conjugated linoleic acid (*cis*-9, *trans*-11 $C_{18:2}$) in milk fat of dairy cows (adapted from Benchaar et al., 2006, 2007).

Only few studies have reported the effects of essential oils and their main components on the fatty acid composition of milk. Supplementing dairy cows with 750 mg/day of MEO did not affect milk fatty acid profile (Benchaar et al., 2007a). Interestingly, higher inclusion rates of MEO (i.e. 2 g/day; Benchaar et al., 2006) enhanced milk fat content of conjugated linoleic acid (*cis*-9, *trans*-11 $C_{18:2}$ isomer; Figure 5).

The effects of essential oils and their main components on ruminant performance are small, which is not surprising considering the equivocal effects of these plant extracts on dry matter intake and rumen fermentation characteristics. In some studies, the greater performance observed when animals were supplemented with essential oils has been related to higher intakes rather to a modification in rumen microbial fermentation and nutrient utilization.

In vitro data suggest that there may be potential to select essential oils that selectively inhibit rumen bacterial populations involved in the process of biohydrogenation of unsaturated fatty acids, which may enhance the concentrations of health-promoting fatty acids (e.g. conjugated linoleic acid) in ruminant derived products. However, further research is required to assess the potential of essential oils to improve fatty acid composition of milk fat.

CONCLUSIONS

The antimicrobial activity of essential oils against a variety of microorganisms has been demonstrated in several studies. Essential oils and their constituents have been shown to inhibit the growth of several pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella* spp. and *Staphylococcus aureus*. This well documented antimicrobial activity of essential oils has recently prompted a plethora of researchers to examine their potential to modify rumen microbial populations to enhance efficiency of rumen fermentation and improve nutrient utilisation in ruminants. The resurgence of interest in using essential oils in ruminant nutrition and production has increased particularly in Europe after the ban on the use of growth-promoting antibiotics, including ionophores, in livestock production.

In ruminant nutrition, the potential of essential oils to favourably alter microbial fermentation has been mostly assessed using batch or continuous culture *in vitro* systems. However, *in vitro* systems have limitations (i.e. difficulty of simulating the diversity and dynamics of microbial populations) and therefore, the ultimate value of essential oils for altering rumen microbial fermentation must be assessed *in vivo*.

Results from *in vitro* studies show that the effects of essential oils and their main components on rumen microbial fermentation are variable and often contradictory, which can be related to differences in doses and the technique used (batch vs. continuous system). It appears that high doses of essential oils are required to alter rumen microbial fermentation and in most cases, the beneficial effects on N metabolism (i.e. reduction in protein degradation and ammonia N production) were counterbalanced by a decrease in total VFA concentration (i.e. feed degradation). When used at low and moderate doses, the effects were negligible, probably due to the adaptation of rumen microbes to essential oils, as has been shown in several continuous culture studies.

The number of published papers on the effects of essential oils and their main components *in vivo* is surprisingly low. Results from the few studies published to date have been only partly convincing and revealed no effects on ruminant performance (milk and growth), which was very often consistent with the lack of effects on rumen microbial fermentation characteristics (pH, VFA, and ammonia N) and diet digestibility. Microbial populations exhibit a remarkable ability

to adapt rapidly to a wide variety of antimicrobial agents such as ionophores, saponins and tannins, and there is some evidence that the rumen microbial population also adapts to continual exposure to essential oils. This adaptive response represents a serious challenge for commercial application of this feed additive technology.

Although little research has been conducted to investigate bacterial resistance to essential oils, some studies have shown that pathogenic bacteria are able to develop resistance to essential oils. Brul and Coote (1999) have reviewed this topic and Nelson (2000) has reported how *Staphylococcus aureus* is able to develop resistance to tea tree (*Melaleuca alternifolia*) essential oil. More research is warranted to determine the capacity of rumen bacteria to develop resistance to essential oils.

TAKE HOME MESSAGE

Over the last few years, the use of plant bioactive compounds for animal health and productivity has been of increasing research interest. This recent surge of interest has been stimulated by the search for alternatives to growth-promoting antibiotics in livestock production. Public concern over the routine use of antibiotics in animal feed has increased in recent years because of their possible contribution to emergence of antibiotic resistant bacteria. Plants produce an array of diverse secondary metabolites such as essential oils that, when extracted and concentrated, may exert antimicrobial activities against a wide variety of microorganisms including bacteria, protozoa, fungi and viruses. Accordingly, considerable research effort has been devoted towards exploiting the antimicrobial properties of essential oils to manipulate rumen microbial fermentation in order to improve nutrient utilization in the animal and reduce the environmental impact of livestock production systems. Most of the studies conducted to date have been laboratory based (i.e. *in vitro*) and of short-term nature. Results from *in vitro* batch and continuous culture studies showed that at high doses, essential oils reduced ammonia nitrogen concentration and methane production, but in many cases this was associated with a decrease in total volatile fatty acid concentration and feed digestion. Evidence for *in vivo* antimicrobial activity of essential oils has been equivocal and to date, *in vivo* studies (effects on rumen fermentation, nutrient utilization, and ruminant performance) have been only partly conclusive. Literature data suggest that rumen microbial populations may adapt when exposed continually to essential oils. Such a response represents a major challenge to developing essential oil feed additives with long-lasting effects. More *in vivo* research is required to fully assess the potential use of essential oils as feed additives in ruminant nutrition.

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