Asian Journal of Animal and Veterinary Advances, 2011
ISSN 1683-9919 / DOI: 10.3923/ajava.2011.

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

Amlan K. Patra
Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, 37 K.B. Sarani, Belgachia, Kolkata, 700037, India

ABSTRACT

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

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

INTRODUCTION

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

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

CHEMISTRY OF ESSENTIAL OILS

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

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

ESSENTIAL OILS ON RUMEN MICROBIAL POPULATIONS

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

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

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

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

ESSENTIAL OILS ON DIGESTIBILITY AND RUMEN FERMENTATION

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

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

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

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

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

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

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

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

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

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

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

lowered methane. However, *in vivo* study of Beauchemin and McGinn (2006) with EO mixture fed to beef cattle (1 g day^{-1}) for 21 days did not reveal any effect on methanogenesis. Similarly, Pinus mugos oil, which has been reported to contain pinene, limonellen, ω3-caren and β-phellandren with proportions of 35, 12, 25 and 14%, respectively, showed no antimethanogenic activity (Soliva *et al.*, 2008) probably due to inclusion of a low dose (8 mg L^{-1}).

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

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

RUMINANT PERFORMANCE

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

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

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

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

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

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

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

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

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

The EO or their metabolic products could be present in milk and meat products due to feeding of EO. For example, feeding of caraway seed and camomile to goat resulted in limonene and carvone (the main EO component of caraway seed) in milk; however, the EO compounds present in camomile were not detected in milk (Molnar *et al*., 1997). Various monoterpenes such as α-pinene, β-pinene, β-mircene, sabinene, camphene, δ3-carene and limonene were present in milk of cows grazing dicotyledons predominated Alpine pasture (Noni and Battelli, 2008; Chion *et al*., 2010). Therefore, the presence of EO or their derivatives could enrich specific organoleptic and nutritional properties to the dairy products that could provide an added value to the product (Chion *et al*., 2010).

CONCLUSIONS

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

ACKNOWLEDGMENT

The research grant provided by Indian Council of Agricultural Research, New Delhi is gratefully acknowledged.

REFERENCES

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

Ando, S., T. Nishida, M. Ishida, K. Hosoda and E. Bayaru, 2003. Effect of peppermint feeding on the digestibility, ruminal fermentation and protozoa. Livest. Prod. Sci., 82: 245-248.

Bampidis, V.A., V. Christodoulou, P. Florou-Paneri, E. Christaki, A.B. Spais and P.S. Chatzopoulou, 2005. Effect of dietary dried oregano leaves supplementation on performance and carcass characteristics of growing lambs. Anim. Feed Sci. Technol., 121: 285-295.

Barton, M.D., 2000. Antibiotic use in animal feed and its impact on human health. Nutr. Res. Rev., 13: 279-299.

Beauchemin, K.A. and S.M. McGinn, 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil and canola oil. J. Anim. Sci., 84: 1489-1496.

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

Benchaar, C., J.L. Duynisveld and E. Charmley, 2006b. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. Can. J. Anim. Sci., 86: 91-96.

Benchaar, C., A.V. Chaves, G.R. Fraser, Y. Yang, K.A. Beauchemin and T.A. McAllister, 2007a. Effects of essential oils and their components on *in vitro* rumen microbial fermentation. Can. J. Anim. Sci., 87: 413-419.

Benchaar, C., H.V. Petit, R. Berthiaume, D.R. Ouellet, J. Chiquette and P.V. Chouinard, 2007b. Effects of essential oils on digestion, ruminal fermentation, ruminal microbial populations, milk production and milk composition in dairy cows fed alfalfa silage or corn silage. J. Dairy Sci., 90: 886-897.

Benchaar, C., S. Calsamiglia, A.V. Chaves, G.R. Fraser, D. Colombatto, T.A. McAllister and K.A. Beauchemin, 2008. A review of plant derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol., 145: 209-228.

Benchaar, C. and P.Y. Chouinard, 2009. Short communication: Assessment of the potential of cinnamaldehyde, condensed tannins and saponins to modify milk fatty acid composition of dairy cows. J. Dairy Sci., 92: 3392-3396.

Bhatti, H.Q., Z. Iqbal, S.A.S. Chatha and I.H. Bukhari, 2007. Variations in oil potential and chemical composition of *Eucalyptus crebra* among different districts of Punjab-Pakistan. Int. J. Agric. Biol., 9: 136-138.

Busquet, M., H. Greathead, S. Calsamiglia, A. Ferret and C. Kamel, 2003. Efecto del extracto de ajo y el cinemaldehido sobre la produccion, composicion y residuos en leche en vacas de alta produccion. ITEA, 24: 756-758.

Busquet, M., S. Calsamiglia, A. Ferret and C. Kamel, 2006. Plant extracts affect *in vitro* rumen microbial fermentation. J. Dairy Sci., 89: 761-771.

Calsamiglia, S., M. Busquet, P.W. Cardozo, L. Castillejos and A. Ferret, 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci., 90: 2580-2595.

Cardozo, P.W., S. Calsamiglia, A. Ferret and C. Kamel, 2005. Screening for the effects of natural plant extracts at different pH on *in vitro* rumen microbial fermentation of a high cocncentrate diet for beef cattle. J. Dairy Sci., 83: 2572-2579.

Cardozo, P.W., S. Calsamiglia, A. Ferret and C. Kamel, 2006. Effects of alfalfa extract, anise, capsicum and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef hrifers fed a high-concentrate diet. J. Anim. Sci., 84: 2801-2808.

Castillejos, L., S. Calsamiglia, A. Ferret and R. Losa, 2005. Effects of a specific blend of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. Anim. Feed. Sci. Technol., 119: 29-41.

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

Chaves, A.V., K. Stanford, L.L. Gibson, T.A. McAllister and C. Benchaar, 2008a. Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance and carcass characteristics of growing lambs. Anim. Feed Sci. Technol., 145: 396-408.

Chaves, A.V., K. Stanford, M.E.R. Dugan, L.L. Gibson, T.A. McAllister, F. Van Herk and C. Benchaar, 2008b. Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance and carcass characteristics of growing lambs. Livest. Sci., 117: 215-224.

Chaves, A.V., M.L. He, W.Z. Yang, A.N. Hristov, T.A. McAllister and C. Benchaar, 2008c. Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. Can. J. Anim. Sci., 89: 97-104.

Chion, A.R., E. Tabacco, D. Giaccone, P.G. Peiretti, G. Battelli and G. Borreani, 2010. Variation of fatty acid and terpene profiles in mountain milk and *Toma piemontese* cheese as affected by diet composition in different seasons. Food Chem., 121: 393-399.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

Estell, R.E., E.L. Fredrikson, M.R. Tellez, K.M. Havstad, W.L. Shupe, D.M. Anderson and M.D. Remmenga, 1998. Effects of volatile compounds on consumption of alfalfa pellets by sheep. J. Anim. Sci., 76: 228-232.

Evans, J.D. and S.A. Martin, 2000. Effects of thymol on ruminal microorganisms. Curr. Microbiol., 41: 336-340.

Fraser, G.R., A.V. Chaves, Y. Wang, T.A. McAllister, K.A. Beauchemin and C. Benchaar, 2007. Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems. J. Dairy Sci., 90: 2315-2328.

Gershenzon, J. and R. Croteau, 1991. Terpenoids. In: Herbivores: Their Interactions with Secondary Plant Metabolites, Rosenthal, G.A. and M.R. Berenbaum (Eds.). Vol. 1, Academic Press, San Diego, CA., pp: 165-219.

Hart, K.J., D.R. Yanez-Ruiz, S.M. Duval, N.R. McEwan and C.J. Newbold, 2008. Plant extracts to manipulate rumen fermentation. Anim. Feed Sci. Technol., 147: 8-35.

Kamra, D.N., A.K. Patra, P.N. Chatterjee, K. Ravindra, A. Neeta and L.C. Chaudhary, 2008. Effect of plant extract on methanogenesis and microbial profile of the rumen of buffalo: A brief overview. Aust. J. Exp. Agric., 48: 175-178.

Kozelov, L., F. Tliev, J. Profirov, I.V.S. Nikolov, G. Ganev, T. Modeva and M. Krasteva, 2001. The effect of supplementing sheep with Ropadiar on digestibility and fermentation in the rumen. Zhivotnov. Dni. Nuki., 3: 152-154.

Kumar, R., D.N. Kamra, N. Agrawal and L.C. Chaudhary, 2009. Effect of eucalyptus (*Eucalyptus globules*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Anim. Nutr. Feed Technol., 9: 237-243.

Kung, Jr.L., P. Williams, R.J. Schmidt and W. Hu, 2008. A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. J. Dairy Sci., 91: 4793-4800.

Lourenço, M., P.W. Cardozo, S. Calsamiglia and V. Fievez, 2008. Effects of saponins, quercetin, eugenol and cinnamaldehyde on fatty acid biohydrogenation of forage polyunsaturated fatty acids in dual-flow continuous culture fermenters. J. Anim. Sci., 86: 3045-3053.

Macheboeuf, D., D.P. Morgavi, Y. Papon, J.L. Mousset and M. Arturo-Schaan, 2008. Dose-response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. Anim. Feed Sci. Technol., 145: 335-350.

Malecky, M., L.P. Broudiscou and P. Schmidely, 2009. Effects of two levels of monoterpene blend on rumen fermentation, terpene and nutrient flows in the duodenum and milk production in dairy goats. Anim. Feed Sci. Technol., 154: 24-35.

McInotch, F.M., P. Williams, R. Losa, R.J. Wallace, D.A. Beever and C.J. Newbold, 2003. Effects of essential oils on ruminal microorhanisms and their protein metabolism. Applied Environ. Microbiol., 69: 5011-5014.

McKay, D.L. and J.B. Blumberg, 2006. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.). Phytother Res., 20: 519-530.

Meyer, N.F., G.E. Erickson, T.J. Klopfenstein, M.A. Greenquist, M.K. Luebbe, P.Williams and M.A. Engstrom, 2009. Effect of essential oils, tylosin and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation and digestibility. J. Anim. Sci., 87: 2346-2354.

Molnar, A., E. Lemberkovices and S. Spiller, 1997. Detection of caraway and camomile components in goat milk. Tejqazdasaq, 57: 22-27.

Newbold, C.J, F.M. McIntosh, P. Williams, R. Losa and R.J. Wallace, 2004. Effects of a spesific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol., 114: 105-112.

Noni, I.D. and G. Battelli, 2008. Terpenes and fatty acid profiles of milk fat and Bitto cheese as affected by transhumance of cows on different mountain pastures. Food Chem., 109: 299-309.

Oh, H.K., T. Sakai, M.B. Jones and W.M. Longhurst, 1967. Effect of various essential oils isolated from douglasfir needles upon sheep and deer rumen microbial activity. Applied Microbiol., 15: 777-784.

Oh, H.K., M.B. Jones and W.M. Longhurst, 1968. Comparison of rumen microbial inhibition resulting from various essential oils isolated from relatively unpalatable plant species. Applied Microbiol., 16: 39-44.

Patra, A.K., 2007. Nutritional management in organic livestock farming for improved ruminant health and production–An overview. Livest. Res. Rural Develop., 19

Patra, A.K. and J. Saxena, 2009a. A review of the effect and mode of action of saponins on microbial population and fermentation in the rumen and ruminant production. Nutr. Res. Rev., 22: 204-219b.

Patra, A.K. and J. Saxena, 2009b. Dietary phytochemicals as rumen modifiers: A review of the effects on microbial populations. Antonie Van Leeuwenhoek, 96: 363-375.

Patra, A.K. and J. Saxena, 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in ruminants. Phytochemistry, 71: 1198-1222.

Patra, A.K., D.N. Kamra and N. Agarwal, 2010. Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds *in vitro*. J. Sci. Food Agric., 90: 511-520.

Russell, J.B., H.J. Strobel and G. Chen, 1988. Enrichment and isolation of a ruminal bacterium with a very high specific activity of ammonia production. Applied Environ. Microbiol., 54: 872-877.

Sallam, S.M.A., I.C.S. Bueno, P. Brigide, P.B. Godoy, D.M.S.S. Vitti and A.L. Abdalla, 2009. Efficacy of eucalyptus oil on *in vitro* rumen fermentation and methane production. Options Mediterraneennes, 85: 267-272.

Santos, M.B., P.H. Robinson, P. Williams and R. Losa, 2010. Effects of addition of an essential oil complex to the diet of lactating dairy cows on whole tract digestion of nutrients and productive performance. Anim. Feed Sci. Technol., 157: 64-71.

Soliva, C.R., S. Widmer and M. Kreuzer, 2008. Ruminal fermentation of mixed diets supplemented with St. Johns Wort (*Hypericum perforatum*) flowers and pine (*Pinus mugo*) oil or mixtures containing these preparations. J. Anim. Feed Sci., 17: 352-362.

Spanghero, M., C. Zanfi, E. Fabbro, N. Scicutella and C. Camellini, 2008. Effects of a blend of essential oils on some end products of *in vitro* rumen fermentation. Anim. Feed. Sci. Technol., 145: 364-374.

Tassoul, M.D. and R.D. Shaver, 2009. Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cows. J. Dairy Sci., 92: 1734-1740.

Tatsouka, N., K. Hara, K. Mlkuni, K. Hara, H. Hashimoto and H. Itabashi, 2008. Effects of the essential oil cyclodextrin complexes on ruminal methane production *in vitro*. Anim. Sci. J., 79: 68-75.

Wallace, R.J., N.R. McEwan, F.M. McInotoch, B. Teferedegne and C.J. Newbold, 2002. Natural products as manipulators of rumen fermentation. Asian Aust. J. Anim. Sci., 10: 1458-1468.

Wallace, R.J., 2004. Antimicrobial properties of plant secondary metabolites. Proc. Nutr. Soc., 63: 621-629.

Wang C.J., S.P. Wang and H. Zhou, 2009. Influences of flavomycin, ropadiar and saponin on nutrient digestibility, rumen fermentation and methane emission from sheep. Anim. Feed Sci. Technol., 148: 157-166.

Yang, W.Z., C. Benchaar, B.N. Ametaj, A.V. Chaves, M.L. He and T.A. McAllister, 2007. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. J. Dairy Sci., 90: 5671-5681.

Yang, W.Z., B.N. Ametaj, C. Benchaar, M.L. He and K.A. Beauchemin, 2010a. Cinnamaldehyde in feedlot cattle diets: Intake, growth performance, carcass characteristics and blood metabolites. J. Anim. Sci., 88: 1082-1092.

Yang, W.Z., B.N. Ametaj, C. Benchaar and K.A. Beauchemin, 2010b. Dose response to cinnamaldehyde supplementation in growing beef heifers: Ruminal and intestinal digestion. J. Anim. Sci., 88: 680-688.