EFFECTS OF GERANIOL AND CAMPHENE ON *IN VITRO* RUMEN FERMENTATION AND METHANE PRODUCTION*

M. Joch[1,2], V. Kudrna[2], B. Hučko[1], M. Marounek[1,2]

[1]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Prague, Czech Republic
[2]Institute of Animal Science, Prague-Uhříněves, Czech Republic

The objective of this study was to determine the effects of geraniol and camphene at three dosages (300, 600, and 900 mg l^{-1}) on rumen microbial fermentation and methane emission in *in vitro* batch culture of rumen fluid supplied with a 60 : 40 forage : concentrate substrate (16.2% crude protein, 33.1% neutral detergent fibre). The ionophore antibiotic monensin (8 mg/l) was used as positive control. Compared to control, geraniol significantly ($P < 0.05$) reduced methane production with increasing doses, with reductions by 10.2, 66.9, and 97.9%. However, total volatile fatty acids (VFA) production and *in vitro* dry matter digestibility were also reduced ($P < 0.05$) by all doses of geraniol. Camphene demonstrated weak and unpromising effects on rumen fermentation. Camphene did not decrease ($P > 0.05$) methane production and slightly decreased ($P < 0.05$) VFA production. Due to the strong antimethanogenic effect of geraniol a careful selection of dose and combination with other antimethanogenic compounds may be effective in mitigating methane emission from ruminants. However, if a reduction in total VFA production and dry matter digestibility persisted *in vivo*, geraniol would have a negative effect on animal productivity.

essential oil, monoterpene, methanogenesis, volatile fatty acids

DE GRUYTER OPEN

doi: 10.1515/sab-2017-0012
Received for publication on February 2, 2016
Accepted for publication on October 6, 2016

INTRODUCTION

Development of mitigation strategies to reduce methane (CH_4) emissions from ruminants is currently the subject of scientific and public interest (J a n s s e n , 2010). Methane produced by ruminants represents a significant energy loss to the animal ranging from 2 to 12% of gross energy intake (J o h n s o n , J o h n s o n , 1995), which might otherwise be available for growth or milk production. Methane production through the enteric fermentation of feed also contributes to the greenhouse gas emissions which have a global warming potential (B o d a s et al., 2012).

Bioactive plant metabolites are an important contemporary research area to create substitutes for chemical feed additives due to their potential to modify rumen fermentation, mainly in terms of reducing methane production and selecting rumen microorganisms (bacteria, protozoa, fungi) to increase feed utilization and volatile fatty acids (VFA) production (C a l a b r ò , 2015). Among plant metabolites, essential oils (EO) have received much attention (B e n c h a a r et al., 2007). Essential oils are aromatic lipophilic compounds with strong antimicrobial activity (C o w a n , 1999) which inhibit growth and survival of most microorganisms, especially bacteria. Based on a possible selective effect on specific ruminal microbial communities, various essential oils have been tested to decrease methanogenesis (B o d a s et al., 2012). The more extensively studied EO (or active compounds of EO), at least regarding their effects on CH_4 production, have been thymol, carvacrol, eugenol, cinnamaldehyde, anethol, and juniper berry and peppermint oils (C a l s a m i g l i a et al., 2007; B e n c h a a r , G r e a t h e a d , 2011). Although a relatively large number of EO have recently been tested for their methane reduction potential, there have not yet been major breakthroughs that could be applied in practice (C i e s l a k et al., 2013).

* Supported by the Ministry of Agriculture of the Czech Republic, Project MZeRO0715.

The objective of this study was to determine the effects of increasing doses of two monoterpenes with proven antimicrobial activity (Scortichini, Rossi, 1991; Chen, Viljoen, 2010), geraniol and camphene, that have not been extensively tested in previous studies, on *in vitro* ruminal fermentation profile. The specific objectives of the study were to determine the effects of these compounds on methane production, total gas production, VFA concentration and proportion, and *in vitro* dry matter digestibility (IVDMD). The ionophore antibiotic monensin was also added as the positive control to compare its effects with those of the EO active compounds in the same *in vitro* conditions.

MATERIAL AND METHODS

Treatments

Experimental treatments were: control (no additive), geraniol (300, 600, and 900 mg l^{-1} of the culture fluid, 98% purity), camphene (300, 600, and 900 mg l^{-1}, 95% purity), and monensin (8 mg l^{-1}; M5273) (all Sigma-Aldrich, USA). Experimental treatments were evaluated in triplicate for each dose of each compound and the control.

Ruminal inoculum

The ruminal inoculum for the *in vitro* incubations was collected from one fistulated lactating Holstein cow (body weight 728 kg; in fifth lactation; milk yield 28.5 kg per day) 2 h after the morning feeding. The total mixed ration (TMR) of cow was composed on a dry matter (DM) basis of corn silage (31.2%), alfalfa silage (19.7%), concentrate mixture (33.0%), ensiled crushed corn cobs with bracts (LKS; 6.0%), brewers grain (8.5%), and wheat straw (1.6%). The concentrate mixture was a commercial mixture for lactating cows (wheat 30.0% DM of mixture, triticale 16.8%, soybean meal 16.5%, rapeseed meal 16.0%, Premin DO1 (VVS Verměřovice s.r.o, Czech Republic) 8.0%, AminoPlus (Ag Processing Inc., USA) 6.5%, C16 (Berg & Schmidt Sdn. Bhd., Malaysia) 5.2%, sodium bicarbonate 1.0%). Cow was fed *ad libitum*. From cow, about 500 ml of rumen sample was collected into a 500 ml capacity vacuum flask, leaving no headspace in the sample flask. The rumen sample was brought to the laboratory, strained through two layers of cheesecloth, and used within 20 min.

Substrate and *in vitro* incubation

The experimental substrate consisted (DM basis) of corn silage (30%), alfalfa silage (30%), and concentrate mixture (40%). The chemical composition per kg DM of the substrate was 915.5 g organic matter, 44.8 g ether extract, 233.1 g starch, 161.6 g crude protein, 331.2 g neutral detergent fibre (NDF), and 215.0 g acid detergent fibre (ADF).

The *in vitro* batch incubation method was as described by Castro-Montoya et al. (2012) with modifications. Briefly, 300 mg of the dried substrate was incubated in 120 ml capacity gastight incubation flasks flushed with CO_2 having 20 ml of a phosphate-bicarbonate buffer according to McDougall (1948) with modifications (per litre of distilled water: 9.8 g $NaHCO_3$; 7 g $Na_2HPO_4 \times 12\ H_2O$; 0.6 g urea; 0.6 g KCl; 0.03 g $CaCl_2$; 0.06 g $MgSO_4 \times 7\ H_2O$; flushed with CO_2 and adjusted to pH 7.0) and 5 ml rumen fluid in a batch culture incubator (SW 22; Julabo, Germany) with shaking (frequency 120 rpm). Before adding the rumen fluid, a stock solution (200 µl) of each additive was added to each flask to reach the desired concentration in 25 ml of the culture fluid. All compounds were dissolved in 99.5% ethanol. The individual stock solution was prepared for each compound and each dose. Fermentation flasks without additives, but containing 300 mg of the substrate and equal volume (200 µl) of ethanol, were used as a control. The flasks were sealed with butyl rubbers plus crimped aluminum seals and incubated at 39°C for 24 h.

Sampling and analysis

At the end of the 24 h of incubation, gas pressure in the flask was measured using a manometer (Tracable; Fisher Scientific, USA) to determine the total gas production. Then, the gas in the headspace of the bottles was analyzed for methane using a gas chromatograph GC 82F (Labio, Czech Republic) equipped with a flame ionization detector, and Stabilwax (Restec, USA) capillary column (15 m × 0.53 mm ID × 0.5 µm), and with hydrogen as the carrier gas. A sample of 100 µl of gas was injected using a 500 µl Pressure-Lok® gastight syringe (Vici Precision Sampling, USA).

After opening the incubation flask, pH was measured (pH 700; Eutech Instruments, Singapore), and 2 ml of incubation medium was collected and centrifuged (6625 *g*, 1 min). Supernatant (64 µl) was mixed with 736 µl of H_2O, 30 µl of internal standard (2-ethylbutyric acid), and 100 µl of 0.3M formic acid and then centrifuged (6625 *g*, 1 min). Samples were stored at 8°C until the VFA analysis using gas chromatography on a Labio GC 82F equipped with a flame ionization detector and capillary column, and with hydrogen as the carrier gas. Briefly, 1 µl was injected; the injector temperature was 200°C, and the inlet pressure was 50 kPa. The temperature program was 75°C at the start of the injection, increased 5°C/min until 80°C (kept for 80 s), then increased 5°C/min until 128°C (kept for 4 s), and then increased 20°C/min until 160°C (kept for 180 s). The detector temperature was 200°C.

Table 1. Effects of geraniol, camphene, and monensin on pH, total gas and methane productions, and on *in vitro* dry matter digestibility

Treatment (mg l^{-1})	pH	Total gas (ml)	Methane (ml)	IVDMD (g kg^{-1})
Control				
0	6.45	74.2	38.1	386.7
Monensin				
8	6.60*	52.6*	21.3*	392.2
Geraniol				
300	6.50	67.4*	34.2*	325.6*
600	6.66*	39.4*	12.6*	344.4*
900	6.92*	1.8*	0.8*	261.1*
Camphene				
300	6.46	72.3	37.4	371.1
600	6.47	67.6*	35.3	344.4*
900	6.50	66.4*	35.3	340.0*
SEM	0.03	4.78	2.72	8.45

IVDMD = *in vitro* dry matter digestibility, SEM = standard error of the mean

*means within a column differ from control ($P < 0.05$)

The IVDMD was determined at 24 h of incubation. The content of each flask was transferred into pre-weighed 50 ml centrifuge tubes, rinsed and centrifuged (500 *g*, 4°C, 10 min). Supernatants were discarded, and precipitates were dried at 55°C for 48 h and weighed to determine DM concentrations for the estimation of IVDMD.

Chemical analysis of substrate

Samples of alfalfa and corn silage were dried in a forced air oven at 65°C. Subsequently, silages and concentrate samples were ground to pass through a 1-mm sieve. Ground samples were stored for chemical analyses. The chemical composition of substrate was determined according to A O A C (2005) for crude protein (CP as 6.25 × N), starch, and ash, and according to A O A C (1995) for ether extracts. The NDF and ADF were measured according to the method of M e r t e n s (2002). The ADF and NDF were assayed with a heat stable amylase and expressed exclusive of residual ash.

Statistical analysis

All data were analyzed using the SAS software (Statistical Analysis System, Version 6.1, 2013) and the treatment responses in total gas and CH_4 production, VFA concentrations, IVDMD, and pH were examined in separate models with treatment as a factor. Each factor had three observations (i.e. separate flasks) and data were analyzed performing one-way analysis of variance. The effects of treatments were compared with those of the control using the Dunnett test. Significant differences were declared at $P < 0.05$.

RESULTS

The effects of additives (geraniol, camphene, and monensin) on pH, total gas and methane productions, and on IVDMD are presented in Table 1. The effects of additives on VFA production and proportion are presented in Table 2. Of the compounds tested, monensin and higher doses of geraniol (600 and 900 mg l^{-1}) changed ($P < 0.05$) pH. Total gas production was significantly decreased ($P < 0.05$) by all doses of the tested compounds with the exception of the lowest dose of camphene (300 mg l^{-1}). Only monensin and camphene (300 mg l^{-1}) did not decrease ($P > 0.05$) IVDMD compared with the control. Relative to the control, geraniol at doses 300, 600, and 900 mg l^{-1} reduced ($P < 0.05$) methane production by 10.2, 66.9, and 97.9%, respectively. Monensin (8 mg l^{-1}) decreased ($P < 0.05$) methane production by 44.1% compared to the control. In all cases, reduction in methane production was accompanied by a reduction ($P < 0.05$) in total VFA (TVFA) concentration. Total VFA concentration was also decreased ($P < 0.05$) at two higher levels of camphene (600 and 900 mg l^{-1}). The addition of geraniol (600 and 900 mg l^{-1}) and monensin decreased ($P < 0.05$) and the addition of camphene (600 and 900 mg l^{-1}) increased ($P < 0.05$) the molar proportion of acetate. The molar proportion of propionate was reduced ($P < 0.05$) by geraniol (300 and 600 mg l^{-1}) and camphene (900 mg l^{-1}), and increased ($P < 0.05$) by monensin. Molar proportion of butyrate was increased ($P < 0.05$) by geraniol (300 and 600 mg l^{-1}) and camphene (900 mg l^{-1}) and reduced ($P < 0.05$) by monensin. At the dose of 600 mg l^{-1} geraniol decreased ($P < 0.05$) and at the dose of 900 mg l^{-1} increased ($P < 0.05$) the molar propor-

Table 2. Effects of geraniol, camphene, and monensin on total VFA production, individual VFA molar proportion, and acetate to propionate molar ratio

Treatment (mg l^{-1})	TVFA (mmol l^{-1})	Acetate	Propionate	Butyrate	Valerate	BCVFA	A/P
		(mol/100 mol TVFA)					
Control							
0	112.77	65.60	17.47	11.96	1.81	2.23	3.75
Monensin							
8	89.32*	59.21*	27.27*	9.66*	1.76	1.63*	2.17*
Geraniol							
300	105.34*	65.74	16.47*	13.31*	1.84	1.84*	3.99*
600	54.14*	54.07*	13.14*	26.42*	2.21*	4.98*	4.11*
900	32.75*	57.63*	17.74	11.18	1.52*	6.00*	3.25*
Camphene							
300	110.96	66.35	17.40	11.69	1.79	1.93	3.81
600	106.08*	67.16*	16.70	11.91	1.80	1.67*	4.30*
900	102.76*	67.34*	14.85*	13.36*	2.10*	1.67*	4.54*
SEM	5.80	1.00	0.82	1.03	0.04	0.34	0.14

TVFA = total volatile fatty acid, BCVFA = branched-chain volatile fatty acid, A/P = acetate/propionate ratio, SEM = standard error of the mean
*means within a column differ from control ($P < 0.05$)

tion of valerate. Geraniol (300 mg l^{-1}), camphene (600 and 900 mg l^{-1}), and monensin (8 mg l^{-1}) decreased ($P < 0.05$) the molar proportion of branched-chain VFA (BCVFA), while geraniol (600 and 900 mg l^{-1}) increased ($P < 0.05$) the proportion of BCVFA compared with the control.

DISCUSSION

Recent comprehensive reviews have indicated that some EO can promote rumen microbial fermentation and approvingly alter rumen metabolism (Calsamiglia et al., 2007; Benchaar et al., 2008). However, EO are complex mixtures of several individual compounds, which makes it difficult to elucidate the precise mechanism of action (Busquet et al., 2005), and identify particular compounds responsible for the main part of the effect on rumen fermentation. Moreover, the concentration of active components in EO can vary widely depending on cultivar, growing condition, or processing methods of oil extraction (Calsamiglia et al., 2007). Thus, in this study, only pure active components of EO were evaluated for their efficacy to mitigate methane production in *in vitro* ruminal culture.

Selective inhibition by EO (and their compounds) of specific microbes, such as methanogens, protozoa, and bacteria, has been reported and was regarded as the main mechanism of EO for manipulating rumen fermentation (McIntosh et al., 2003). Generally, it is assumed that Gram-positive bacteria are more inhibited by EO than Gram-negative due to their simple cell membrane compared to the more complex cell wall of Gram-negative bacteria (Cimanga et al., 2002).

Geraniol is an acyclic monoterpene alcohol with the chemical formula $C_{10}H_{18}O$. It is a common constituent of several EO and occurs in *Monarda fistulosa* (> 95%), ninde oil (66.0%), rose oil (44.4%), palmarosa oil (53.5%), and citronella oil (24.8%) (Chen, Viljoen, 2010). Geraniol has been shown to have antimicrobial activity against both Gram-positive and Gram-negative bacteria (Dorman, Deans, 2000). In a previous *in vitro* study, when tested in a dosage 500 mg l^{-1}, geraniol reduced total gas and TVFA production, suggesting a reduction in diet digestibility, but did not inhibit methane production (Pirondini et al., 2015). In our study, compared to the control, all doses of geraniol (300, 600, and 900 mg l^{-1}) decreased total gas and TVFA production. This is consistent with the adverse effects of geraniol on IVDMD (Table 1). But all doses of geraniol also inhibited methane production. The highest dose (900 mg l^{-1}) of geraniol reduced methane production by 97.9% compared with the control. Although the results indicate that methane reduction by geraniol was achieved through overall inhibition of fermentation process, at least part of the effect of geraniol on methane production could be due to direct inhibition of methanogenic archaea. Archaea (methanogens) have unique membrane lipids that contain glycerol joined by ether linkages to long chain isoprenoid alcohols. Mevalonate is a key precursor for isoprenoid synthesis by methanogens. Mevalonate is produced by reduction of hydroxymethylglutaryl-SCoA

(HMG-CoA) (Wolin, Miller, 2006). As described by Polo, Bravo (2006), geraniol inhibits HMG-CoA reductase activity, thus geraniol would be expected to specifically inhibit growth of rumen methanogens by inhibiting their synthesis of mevalonate.

Camphene is a bicyclic monoterpene with a pungent smell. It constitutes a minor part of many EO including turpentine oil, cypress oil, citronella oil, ginger oil etc. (Tiwari, Kakkar, 2009). Camphene exerts antimicrobial activity (Scortichini, Rossi, 1991). According to Busquet et al. (2006) ginger oil (3, 30, 300, and 3000 mg l^{-1}) influenced neither TVFA nor a proportion of individual volatile fatty acid in *in vitro* rumen fermentation. Similarly, Hristov et al. (2008) reported no effect of citronella oil (10 and 100 mg l^{-1}) on TVFA and volatile fatty acid proportion. However, camphene is only a minor component of both ginger and citronella essential oil (Tiwari, Kakkar, 2009). Oh et al. (1967) observed that camphene and other monoterpene hydrocarbons slightly promoted the activity of rumen microorganisms in *in vitro* experiment with sheep rumen fluid. To our best knowledge, there are no other reports on the effects of camphene on rumen microbial fermentation. Camphene at 600 and 900 mg l^{-1} reduced TVFA concentrations (by 5.9 and 8.9%, respectively), total gas production, and IVDMD, suggesting that these doses were slightly toxic to rumen bacteria. Methane production was not significantly influenced by camphene. The effects of two higher doses of camphene on the proportion of individual volatile fatty acid were unfavourable (increasing acetate and butyrate and decreasing propionate), except for BCVFA. BCVFA are derived from amino acid catabolism in the rumen (Busquet et al., 2006). The observed reduction in BCVFA in the present trial suggests that at 600 and 900 mg l^{-1} camphene reduced amino acid deamination. Inhibition of amino acid deamination has practical implications because it may increase ruminal escape of dietary protein and overall improve nitrogen metabolism in the rumen and/or animal (Nagaraja et al., 1997).

In the present study, as expected, monensin decreased acetate and butyrate and increased propionate formation. Monensin alters the rumen fermentation by inhibiting species that are prominent in producing acetate, butyrate, and H_2 (Nagaraja et al., 1997). Like the geraniol, monensin also reduced methane production, but different effects on volatile fatty acids proportion suggest that the mechanism of action may not be the same. An explanation may be the fact that monensin targets primarily Gram-positive bacteria (Russell, Strobel, 1989), whereas geraniol have been shown to have a broad spectrum of activity against both Gram-positive and Gram-negative bacteria (Dorman, Deans, 2000). Moreover, methanogenic archaea, in general, are resistant to the antibiotic. Monensin inhibits methanogenesis indirectly by lowering the availability of hydrogen and formate (the primary substrates for methanogens) (Chen, Wolin, 1979). Addition of monensin also resulted in a decrease in TVFA concentration and total gas production without decreasing IVDMD. These results suggest that although monensin could have detrimental effects on the production of TVFA for some rumen bacteria, the overall digestion of the feed was unaffected. The effects of monensin on TVFA concentration varied among *in vitro* studies, with no effect (Durmic et al., 2014), increasing (Castillejos et al., 2008) or decreasing (Chaves et al., 2008) effect on TVFA concentration.

Although geraniol inhibited overall rumen fermentation in our *in vitro* short-term batch experiment, this effect could be only transient. As previously described by Busquet et al. (2005), in contrast with *in vitro* batch fermentation the same doses of certain EO in the long-term continuous culture study were not detrimental to rumen microbial fermentation. The lack of detrimental effects in the long-term continuous culture study could be due to the longer adaptation time allowed to the rumen microflora, which may allow replacement of the inhibited microbial population by other resistant bacterial groups (Busquet et al., 2005). Similar effects have been observed with monensin when comparing its short-term effects *in vitro* vs long-term effects *in vivo* (Schelling, 1984).

CONCLUSION

In summary, this study showed that geraniol can significantly decrease methane production, but also (especially at higher doses) exert adverse effects on ruminal feed digestion and fermentation. The mode of antimethanogenic action of geraniol seems to be different than that of monensin. A combination of geraniol, especially at low doses, with other antimethanogenic agents may be effective in mitigating methane emission from ruminants. However, further research is required to asses *in vivo* effects of geraniol, because if the adverse effect on ruminal feed digestion was persistent *in vivo*, geraniol may not be beneficial for ruminant nutrition. Our results indicate that camphene is not a potent rumen fermentation modifier.

ACKNOWLEDGEMENT

The authors acknowledge K. Ječmínková and L. Čermák for their technical assistance.

REFERENCES

AOAC (1995): Official Methods of Analysis of AOAC International. 16th Ed. Association of Official Analytical Chemists, Arlington, USA.

AOAC (2005): Official Methods of Analysis of AOAC International. 18th Ed. Association of Official Analytical Chemists, Arlington, USA.

Benchaar C, Greathead H (2011): Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Animal Feed Science and Technology, 166–167, 338–355. doi: 10.1016/j.anifeedsci.2011.04.024.

Benchaar C, Chaves AV, Fraser GR, Beauchemin KA, McAllister TA (2007): Effects of essential oils and their components on *in vitro* rumen microbial fermentation. Canadian Journal of Animal Science, 87, 413–419. doi: 10.4141/CJAS07012.

Benchaar C, Calsamiglia S, Chaves AV, Fraser GR, Colombatto D, McAllister TA, Beauchemin KA (2008): A review of plant-derived essential oils in ruminant nutrition and production. Animal Feed Science and Technology, 145, 209–228. doi: 10.1016/j.anifeedsci.2007.04.014.

Bodas R, Prieto N, García-González R, Andrés S, Giráldez FJ, López S (2012): Manipulation of rumen fermentation and methane production with plant secondary metabolites. Animal Feed Science and Technology, 176, 78–93. doi: 0.1016/j.anifeedsci.2012.07.010.

Busquet M, Calsamiglia S, Ferret A, Carro MD, Kamel C (2005): Effect of garlic oil and four of its compounds on rumen microbial fermentation. Journal of Dairy Science, 88, 4393–4404. doi: 10.3168/jds.S0022-0302(05)73126-X.

Busquet M, Calsamiglia S, Ferret A, Kamel C (2006): Plant extracts affect *in vitro* rumen microbial fermentation. Journal of Dairy Science, 89, 761–771. doi: 10.3168/jds.S0022-0302(06)72137-3.

Calabrò S (2015): Plant secondary metabolites. In: Puniya AK, Singh R, Kamra DN (eds): Rumen microbiology: from evolution to revolution. Springer India, New Delhi, 153–159.

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A (2007): Invited review: Essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science, 90, 2580–2595. doi: 10.3168/jds.2006-644.

Castillejos L, Calsamiglia S, Martín-Tereso J, Ter Wijlen H (2008): *In vitro* evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets. Animal Feed Science and Technology, 145, 259–270. doi: 10.1016/j.anifeedsci.2007.05.037.

Castro-Montoya J, De Campeneere S, Van Ranst G, Fievez V (2012): Interactions between methane mitigation additives and basal substrates on *in vitro* methane and VFA production. Animal Feed Science and Technology, 176, 47–60. doi: 10.1016/j.anifeedsci.2012.07.007.

Chaves AV, He ML, Yang WZ, Hristov AN, McAllister TA, Benchaar C (2008): Effects of essential oils on proteolytic , deaminative and methanogenic activities of mixed ruminal bacteria. Canadian Journal of Animal Science, 88, 117–122. doi: 10.4141/cjas07061.

Chen M, Wolin MJ (1979): Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. Applied and Environmental Microbiology, 38, 72–77.

Chen W, Viljoen AM (2010): Geraniol – a review of a commercially important fragrance material. South African Journal of Botany, 76, 643–651. doi: 10.1016/j.sajb.2010.05.008.

Cieslak A, Szumacher-Strabel M, Stochmal A, Oleszek W (2013): Plant components with specific activities against rumen methanogens. Animal: An International Journal of Animal Bioscience, 7 (Suppl. 2), 253–265. doi: 10.1017/S1751731113000852.

Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck A (2002): Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of Ethnopharmacology, 79, 213–220. doi: 10.1016/S0378-8741(01)00384-1.

Cowan MM (1999): Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12, 564–582.

Dorman HJD, Deans SG (2000): Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88, 308–316. doi: 10.1046/j.1365-2672.2000.00969.x.

Durmic Z, Moate PJ, Eckard R, Revell DK, Williams R, Vercoe PE (2014): *In vitro* screening of selected feed additives, plant essential oils and plant extracts for rumen methane mitigation. Journal of the Science of Food and Agriculture, 94, 1191–1196. doi: 10.1002/jsfa.6396.

Hristov AN, Ropp JK, Zaman S, Melgar A (2008): Effects of essential oils on *in vitro* ruminal fermentation and ammonia release. Animal Feed Science and Technology, 144, 55–64. doi: 10.1016/j.anifeedsci.2007.09.034.

Janssen PH (2010): Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Animal Feed Science and Technology, 160, 1–22. doi: 10.1016/j.anifeedsci.2010.07.002.

Johnson KA, Johnson DE (1995): Methane emissions from cattle. Journal of Animal Science, 73, 2483–2492. doi: 1995.7382483x.

McDougall EI (1948): Studies on ruminant saliva: 1. The composition and output of sheep's saliva. The Biochemical Journal, 43, 99–109. doi: 10.1042/bj0430099.

McIntosh FM, Williams P, Losa R, Wallace RJ, Beever DA, Newbold CJ (2003): Effects of essential oils on ruminal microorganisms and their protein metabolism. Applied and Environmental Microbiology, 69, 5011–5014. doi: 10.1128/AEM.69.8.5011-5014.2003.

Mertens D (2002): Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International, 85, 1217–1240.

Nagaraja TG, Newbold CJ, van Nevel CJ, Demeyer DI (1997): Manipulation of ruminal fermentation. In: Hobson PN,

Stewart CS (eds): The rumen microbial ecosystem. Springer Netherlands, Dordrecht, 523–632.

Oh HK, Sakai T, Jones MB, Longhurst WM (1967): Effect of various essential oils isolated from douglas fir needles upon sheep and deer rumen microbial activity. Applied and Environmental Microbiology, 15, 777–784.

Pirondini M, Colombini S, Malagutti L, Rapetti L, Galassi G, Zanchi R, Crovetto GM (2015): Effects of a selection of additives on *in vitro* ruminal methanogenesis and *in situ* and *in vivo* NDF digestibility. Animal Science Journal, 86, 59–68. doi: 10.1111/asj.12249.

Polo MP, Bravo MG (2006): Effect of geraniol on fatty-acid and mevalonate metabolism in the human hepatoma cell line Hep G2. Biochemistry and Cell Biology, 84, 102–111. doi: 10.1139/o05-160.

Russell JB, Strobel HJ (1989): Effect of ionophores on ruminal fermentation. Applied and Environmental Microbiology, 55, 1–6.

Schelling GT (1984): Monensin mode of action in the rumen. Journal of Animal Science, 58, 1518–1527. doi: 10.2134/jas1984.5861518x.

Scortichini M, Rossi MP (1991): Preliminary *in vitro* evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill) Winslow *et al*. Journal of Applied Bacteriology, 71, 109–112. doi: 10.1111/j.1365-2672.1991.tb02963.x.

Tiwari M, Kakkar P (2009): Plant derived antioxidants – geraniol and camphene protect rat alveolar macrophages against t-BHP induced oxidative stress. Toxicology in Vitro, 23, 295–301. doi: 10.1016/j.tiv.2008.12.014.

Wolin MJ, Miller TL (2006): Control of rumen methanogenesis by inhibiting the growth and activity of methanogens with hydroxymethylglutaryl-SCoA inhibitors. International Congress Series, 1293, 131–137. doi: 10.1016/j.ics.2006.01.031.

Corresponding Author:

Ing. Miroslav J o c h , Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Kamýcká 129, 165 21 Prague 6-Suchdol, Czech Republic, phone: +420 224 382 669, e-mail: joch@af.czu.cz