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Evaluation of different essential oils in modulating methane and ammonia production, rumen fermentation, and rumen bacteria *in vitro*

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ABSTRACT

Various essential oils (EO) have been individually evaluated to mitigate methane and ammonia production by rumen microbiota. Interactions between EO can affect their potency but such interactions largely remain unexplored. In the present study, EO from oregano, rosemary, Ceylon cinnamon, cinnamon leaves, cinnamon bark, dill seeds, and eucalyptus were chemically characterized and then evaluated *in vitro*, both individually (at 1.125 ml/L culture) and in three-way EO combinations (at total EO 0.8 ml/L, equal ratio), for their effects on fermentation, methanogenesis, ammoniagenesis, and bacteria and archaea. All the EO and their combinations decreased production of total gas ($P < 0.001$), methane ($P < 0.001$), and ammonia (except eucalyptus EO; $P < 0.001$), but they (except the Ceylon cinnamon-dill seeds-eucalyptus EO combination) also decreased dry matter digestibility ($P < 0.001$). The EO individually decreased the abundances of *Prevotella* spp. ($P < 0.001$) but only oregano EO reduced the abundance of archaea ($P < 0.001$). The EO combinations significantly decreased the abundances of archaea ($P < 0.001$), protozoa ($P < 0.001$), and select groups or species of different rumen bacteria to different extents. Changes in bacterial and archaeal communities in response to several EO combinations were also shown by DGGE analyses. Combination of EO from Ceylon cinnamon, dill seeds, eucalyptus, and probably others, at low concentrations may be a practical approach to mitigate methane emission and nitrogen excretion from ruminant without adverse effect on feed digestion or fermentation.

Abbreviations: A/P, acetate/propionate; CCB, Ceylon cinnamon bark; CIB, cinnamon bark; CIL, cinnamon leaves; CTR, control; DGGE, denaturing gradient gel electrophoresis; DIL, dill seeds; DM, dry matter; DMD, dry matter degradability; EO, essential oils; GHG, greenhouse gas; EUC, eucalyptus leaves; FID, flame ionization detector; GC, gas chromatography; GC–MC, gas chromatography–mass spectroscopy; NDF, neutral detergent fiber; NDFD, neutral detergent fiber degradability; ORE, oregano leaves; PCA, principal component analysis; RI, linear retention indices; ROS, rosemary leaves; SEM, standard error means; VFA, volatile fatty acids.

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1. Introduction

Ruminant livestock production contributes significantly to worldwide greenhouse gas (GHG) emissions. About 71% of the GHG emissions comes as methane produced from enteric fermentation (especially rumen fermentation), followed by nitrous oxide (25%) and methane (4%) from manure (FAO, 2013). In the coming years, the rapid growth of world population will increase the demand for milk and meat by 22% and 14%, respectively (FAO, 2013). This will inevitably increase GHG emissions from livestock production.

Numerous nutritional strategies have been evaluated in recent years to mitigate methane emission and nitrogen excretion from cattle by modulating the ruminal microbial fermentation processes. Some promising results have been obtained from natural feed additives (Hristov et al., 2013; Knapp et al., 2014). Among these natural rumen modifiers, plant secondary metabolites, such as saponins, tannins, and essential oils (EO), are of potential application.

Essential oils are synthetized by many plants as protectants against insect predation and microbial infection, and they are generally considered as safe. The major compounds identified in EO include monoterpene hydrocarbons (e.g. α-pinene, α-phellandrene, *p*-cymene, *m*-cymene, γ-terpinene, and limonene) and phenolic compounds (e.g. carvacrol, thymol, and eugenol). Owing to the phenolic ring and hydroxyl group, phenolic compounds have stronger antimicrobial activities than monoterpene hydrocarbons (Benchaar and Greathead, 2011; Cobellis et al., 2016). Generally, Gram-positive bacteria are more sensitive to EO than Gram-negative bacteria, but small EO compounds, such as carvacrol, are able to interact with cell membrane of Gram-negative bacteria, leading to loss of cell content and cell lysis (Benchaar and Greathead, 2011; Cobellis et al., 2016). Eugenol, a phenolic compound of cinnamon EO, can inactivate some microbial enzymes (Burt, 2004; Benchaar and Greathead, 2011). The strong antimicrobial activity of cinnamaldehyde, similar to that of carvacrol, has been attributed to the presence of a carbonyl group, which was thought to be able to disrupt microbial cell membrane and inactivate microbial enzymes (Benchaar and Greathead, 2011; Cobellis et al., 2016). However, unlike the antimicrobial activity exhibited by their individual compounds, the antimicrobial potency of EO varies considerably depending on chemical composition (both components present and their proportions), chemical configurations of components, and interactions among EO components (Burt, 2004). For example, cinnamon EO is more anti-methanogenic than its individual compounds, which suggests a synergic activity among its components (Macheboeuf et al., 2008). Combinations of different EO could also bring about a greater antimicrobial efficiency due to additive and/or synergistic effects that can occur between components of different EO (Benchaar and Greathead, 2011).

Recently, many EO have been shown to positively affect starch and protein degradation, production of ammonia, volatile fatty acids (VFA), and methane due to their antimicrobial activities against some ruminal microorganisms, such as methanogenic archaea and hyper-ammonia producing bacteria (Patra, 2011). However, these positive effects are often accompanied with negative effects on fiber degradation (Patra and Yu, 2012). In fact, due to both the complexity of the rumen microbial ecosystem and EO composition, the effects of EO on different ruminal microbial populations and on their interactions with feed fermentation seem to be difficult to predict. Mixed responses of rumen microbiome to EO were also reported in different studies with respect to methane production, fermentation characteristics, and microbial populations. The chemical composition of EO, which affects their antimicrobial activities, is highly variable depending on many factors (e.g. plant species, stage of growth, parts of plant, extraction method). To date, only a few studies evaluated EO with a known chemical composition in modulating rumen microbiome and function.

In the present study, we determined the chemical composition of seven EO and comparatively evaluated their effects on methane and ammonia production, feed degradability, VFA production, rumen bacterial and archaeal communities, and abundance of common rumen microbial groups *in vitro*. This study showed that combinations of certain EO could potentially decrease methane emissions from cattle with little or no detrimental effect on feed digestion or fermentation.

2. Methods

2.1. Essential oils and experimental design

Essential oil from oregano leaves (ORE; *Thymus capitatus* L.), rosemary leaves (ROS; *Rosmarinus officinalis* L.), Ceylon cinnamon bark (CCB; *Cinnamomum zeylanicum*), cinnamon leaves (CIL; *Cinnamomum zeylanicum* Blume), cinnamon bark (CIB; *Cinnamomum zeylanicum* Blume), dill seeds (DIL; *Anethum graveolens* L.), and eucalyptus leaves (EUC; *Eucalyptus globulus* Labill.) were purchased from Essential Srl (Montopoli Val d'Arno, Italy). The composition of the EO was determined by gas chromatography (GC) and by gas chromatography–mass spectrometry (GC–MC) that were controlled by the HP ChemStation Software. The GC instrument (HP 6890) was equipped with a MS 5973 mass selective detector (Hewlett Packard, Palo Alto, CA), a fused silica capillary column (HP-5MS; 30 m × 0.25 mm i.d., 0.25 μm film thickness) and a flame ionization detector (FID). The oven temperature was programmed to hold at 40 °C for 7 min, ramp to 270 °C at 10 °C/min, and hold at 270 °C for 20 min. Injector and detector temperatures were maintained at 250 and 270 °C, respectively. Each of the EO samples was diluted in hexane to a final concentration of 0.125 μl/ml, and 1 μl was injected into the GC in the splitless mode using helium as carrier gas. Each component was calculated by dividing the peak area of each component by the total area of all the components detected. The values were the mean of 3 injections of each EO sample. All compounds were identified by comparison of their linear retention indices (RI) relative to the retention times of a homologous series of C5–C20 alkanes reported in the literature (Adams, 2007) and by comparison to the mass spectra from the NIST98 Mass Spectral Database.