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In vivo and *in vitro* effects of a blend of essential oils on rumen methane mitigation

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ABSTRACT

The effect of Agolin Ruminant, a blend of essential oils, on methane (CH_4) emissions were investigated in two *in vivo* experiments and in four *in vitro* experiments. In the *in vivo* experiments, four lactating dairy cows and four beef heifers were supplemented 0.2 g/d of the essential oils (*ca.* 2–4 ppm *m*/*v*) during an eight-weeks period, where the first two weeks served as control (no essential oils supplementation). In dairy cattle, essential oils tended to decrease the daily CH_4 emissions (g/d) and CH_4 relative to dry matter intake (g/kg DMI) by 15% and 14%, respectively, after 6 weeks of supplementation ($P=0.07$), but no difference was observed for CH_4 relative to milk production (g/kg milk) ($P=0.64$) or CH_4 relative to bodyweight (g/kg BW) ($P=0.12$). In the *in vivo* experiment with beef cattle daily CH_4 emissions and CH_4 relative to DMI did not change when supplemented the essential oils at a dose of 0.2 g/d (numerical decreases of 10 and 11% for g CH_4/d and g CH_4/kg DMI, respectively) but CH_4 relative to body weight tended to decrease by 20% after 6 weeks of supplementation ($P=0.07$). The *in vitro* experiments were expected to replicate the results observed *in vivo*. However, no decrease in CH_4 production was observed in 24 h batch incubations at concentrations up to 30 ppm (*m*/*v*). A longer contact time between the essential oils (15 and 30 ppm) and the feedstuff (essential oils added *ca.* 16 h prior the start of the incubation) did not elicit any effect on CH_4 production and was not different from addition immediately prior to the start of the incubation. Longer incubation time (96 h and 14 d) and regular supply of both substrate and additive in a consecutive batch incubation system did not induce CH_4 inhibition up to essential oils doses of 30 ppm (*m*/*v*) and hence, also were not able to replicate *in vivo* results. Using the gas production technique (GPT) methane was inhibited by 17% with an essential oils dose of 30 ppm after 24 h, but this decrease was not constant across all times during the 72 h incubation. The blend of essential oil was effective reducing daily emissions of methane in dairy cattle and emissions relative to body weight in beef cattle, interestingly, these effects were not observed *in vitro* regardless of the techniques used to replicate *in vivo* results. This might be due to differences in the mode of action of the essential oils *in vitro* and *in vivo*, which merits attention for future research.

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1. Introduction

In 2012, the agricultural sector worldwide emitted around 5.4 billion tons (CO_2 eq.) greenhouse gases, with enteric fermentation representing around 39% of those emissions (FAOSTAT, 2014). Methane emissions from agricultural activities represented 77% of the total Belgian methane emissions in 2010 (UNFCCC, 2011a), with 68% of agricultural methane originating from enteric fermentation (UNFCCC, 2011b). Therefore, it exists a global interest to decrease methane (CH_4) emissions from ruminants. In this regard, antimicrobial compounds like, ionophores are known to have anti-methanogenic effects in dairy and beef cattle, with variable results in the magnitude of the inhibition and the persistence of their inhibitory effects (Beauchemin et al., 2008). However, the prohibition to use antibiotics in animal feeding in Europe has

Abbreviations: BW, body weight; DMI, dry matter intake; GPT, gas production technique; VFA, volatile fatty acids

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prompted the search for alternatives to these products and the research on plant derived compounds to modulate ruminal fermentation. In general, a large number of plant compounds are screened *in vitro*, and the results from promising compounds are later validated *in vivo* (Beauchemin et al., 2008).

Essential oils are plant secondary metabolites believed to have potential as dietary additives, due to their strong antibacterial properties (Burt, 2004). Essential oils inhibited the energy metabolism of *Streptococcus bovis* and *Selenomonas ruminantium* (Evans and Martin, 2000) and the growth of *Methanobrevibacter smithii*, a rumen Archaea (McIntosh et al., 2003). The latter is of major interest given worldwide attempts to decrease CH_4 from ruminants.

Essential oils have been studied both *in vivo* and *in vitro*, but effects on CH_4 were variable, which may be linked to the large diversity in the nature of these compounds (Calsamiglia et al., 2007). Hence, routine screening is required to assess the effectiveness of numerous essential oils and blends at different doses. *In vitro* batch incubation systems are practical tools for this purpose. However, in some cases *in vitro* observations do not reflect the results found *in vivo* (Flachowsky and Lebzien, 2012), making it challenging to transform effective *in vitro* concentrations to *in vivo* doses. Given the current interest on rumen CH_4 production, the focus of this study is to test the effects of a blend of essential oils on CH_4 production *in vivo* with both beef and dairy cattle, and *in vitro* using different techniques.

2. Materials and methods

Procedures with animals were approved by the ethical commission of the Institute for Agricultural and Fisheries Research (ILVO), Belgium (Reference number EC 2011-154).

2.1. Materials

Additive. The commercial blend of essential oils provided by AGOLIN SA (Bière, Switzerland) contained 200 g/kg (m/m) of active compounds, mainly coriander oil, geranyl acetate and eugenol. From now onwards when referring to doses and concentrations, we will mean the amount of active compounds supplemented (*e.g.* 0.2 g of essential oils, when the amount of the commercial blend of essential oils added was 1 g).

2.2. In vivo experiments

2.2.1. Blend of essential oils experiment with dairy cattle

Four multiparous lactating Holstein dairy cows with an average body weight of 603 kg (±70.0) and being 296 (±97.6) days in milk at the start of the experiment were used for the *in vivo* measurements. The experiment ran over a period of eight-weeks and was performed between October and December 2011.

Before the start of the experiment, cows had *ad libitum* access to a mixture of grass silage (460 g/kg DM), maize silage (370 g/kg DM) and soybean meal (50 g/kg DM) and were supplemented with concentrate (120 g/kg DM) (Table 1). The proportions of the offered feedstuffs during this period were equal to those during the experiment. Before the start of the experiment a fixed amount (95% of the *ad libitum* intake of the cow with the lowest intake) was offered daily until the end of the measurements. This was done to avoid confounding effects (*e.g.* treatment and advanced lactation stage) on feed intake and passage rate in the rumen. The proportions of feedstuffs along with their chemical composition are presented in Table 1. The first two weeks of the experiment the animals were fed the basal diet (Control period); the blend of essential oils was fed starting on the third week of the experiment. Cows were fed and milked twice a day at 0730 AM and 0530 PM and had free access to drinking water at every time throughout the experiment.

2.2.2. Blend of essential oils experiment with beef cattle

Four Belgian Blue double muscled beef heifers with an average body weight of 484 kg (±111.3 kg) at the start of the experiment were used for the *in vivo* measurements. The eight-week experiment was performed between October and December 2012.

Two weeks before the experiment, the animals were fed maize silage *ad libitum* and supplemented with concentrate. The proportions (Table 1) during this period were equal to those during the experiment. Similar to the trial with dairy cattle, before the start of the experiment, a fixed amount (95% of the *ad libitum* intake of the heifer with the lowest intake) was offered daily until the end of the experiment. The first two weeks of the experiment the animals were fed the basal diet (Control period); the blend of essential oils was fed starting on the third week of the experiment. Heifers were fed once daily at 0730AM and had free access to drinking water throughout the experiment.

2.2.3. Blend of essential oils supplementation

For both beef and dairy cattle the blend of essential oils was supplemented daily starting after the gas measurements of the

Table 1
Ingredient and chemical composition of the experimental diets (g/kg DM) as offered to both dairy cows and beef heifers.

	Dairy cattle				Beef cattle	
	Grass silage	Maize silage	Concentrate	Soybean meal	Maize silage	Concentrate
Dietary proportion (g/kg DM)	460	370	120	50	500	500
Chemical composition (g/kg DM)						
Dry matter (g/kg fresh matter)	313	376	877	862	373	876
Crude protein	171	83	189	516	71	212
Crude fat	43	29	31	33	38	79
Crude ash	100	41	84	64	65	76
Sugars	25	0.4	105	109	n.d.	83
Starch	n.d.	418	219	n.d.	382	34
Neutral detergent fiber	319	458	208	158	346	268
Acid detergent fiber	178	286	135	122	195	155
Acid detergent lignin	13.3	16.6	37.5	37.0	15.8	21.6
Organic matter	900	959	916	936	965	924
Organic matter digestibility (g/kg DM)[a]	799	787	898	907	753	812

[a] *In vivo* organic matter digestibility estimated from cellulose digestibility.

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