The effects of individual or blended essential oils on rumen gas production and ammonia accumulation *in vitro*

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Introduction The incorporation of essential oils into ruminant diets has the potential to beneficially modify rumen fermentation. In the literature, essential oils have been reported to alter several rumen parameters such as molar proportions of volatile fatty acids (VFA), methane production and amino acid degradation (reviewed by Calsamiglia *et al*. 2007). The majority of studies that have investigated the effects of essential oils have largely focused on inclusion levels of individual oils. Few studies have investigated the potential synergistic effects of blending different essential oils. The aim of this study was to analyse the effects of three essential oils, eugenol, limonene and terpinene and their respective blends on rumen fermentation *in vitro*.

Materials and methods A 70:30 grass hay (*Lolium perenne)* and concentrate basal diet milled through a 1 mm screen, was incubated in 125ml serum bottles. Incubation buffer (90 ml), prepared according to Theodorou *et al.* (1994), and 10 ml of strained rumen fluid were added to each bottle. The rumen fluid used was sourced from three sheep rumens obtained from a local abattoir. Essential oils were added to the bottles to achieve a dosage level of 500 mg/litre either as individual or blended (50:50) mixes of essential oils. The seven experimental treatments were as follows: control (CON, unsupplemented diet), eugenol (EUG, 100%), limonene (LIM, 100%), terpinene (TER, 100%), eugenol + limonene (EuLi, 50:50), limonene + terpinene (LiTe, 50:50), terpinene + eugenol (TeEu, 50:50). Four replicates of each treatment were incubated and gas production was measured at 3, 6, 12, 24, 48 and 72 hours using a pressure transducer (Mauricio, *et al* 1999). Another set of extra bottles, treated as above, were incubated alongside and stopped after 12, 24, 48 and 72 hours and their contents analysed for ammonia nitrogen (NH_3-N) and VFA. Treatment effects were analysed using analysis of variance with Genstat 11th Edition.

Results Gas production data are summarised in Table 1. After 3, 6, 12 and 24 hours of incubation all essential treatments significantly reduced gas production compared to the control (P<0.001). Blending terpinene and eugenol (TeEu) consistently caused the greatest reduction in gas production compared to other treatments at all times (P<0.001). Other essential oil blends (EuLi and LiTe) generally did not alter gas production compared to individual supplements of these essential oils. The effects of essential oils on NH_3-N concentration are shown in Table 2. There was no difference in NH_3-N after 12 hours of incubation across all treatments. In general, all essential oil treatments reduced NH_3-N concentration at 24 and 48 hours compared to the control. LIM elicited the most significant reduction in NH_3-N at 24 hours.

Table 1 Effects of either individual or combinations (50:50) of essential oils on cumulative gas production (ml/g DM) *in vitro* (n=4)

	Experimental Treatments								
Time (hr)	CON	EUG	LIM	TER	EuLi	LiTe	TeEu	sed	*P*-value
3	31	19	21	23	18	23	16	1.4	P<0.001
6	60	39	40	41	35	44	33	2.8	P<0.001
12	98	72	66	72	64	72	57	5.1	P<0.001
24	138	118	83	91	90	88	82	4.7	P<0.001
48	178	174	116	116	117	109	104	7.2	P<0.001
72	193	193	136	134	132	126	114	8.0	P<0.001

Table 2 Effects of either individual or combinations (50:50) of essential oils on NH_3-N (mg/litre) concentration *in vitro*

	Experimental Treatments								
Time (hr)	CON	EUG	LIM	TER	EuLi	LiTe	TeEu	sed	*P*-value
12	242	256	258	259	250	253	262	5.7	NS
24	294	308	272	273	281	283	288	6.9	P=0.001
48	383	343	296	302	318	297	316	13.2	P=0.002
72	422	352	333	328	354	343	359	8.2	P<0.001

Conclusion This study demonstrates that essential oils have antimicrobial activities that depress both gas production and NH_3-N concentration compared to unsupplemented controls. Although essential oil combinations reduced gas production they appear not to be as effective as individual unblended essential oils at reducing ammonia production *in vitro*.

References

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