In vitro evaluation of the potential of plant-based anti-methanogenic feed additives and metal ions for rumen fermentation

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Enteric methane emitted by ruminants is both a significant energy loss from the animal and a major source of greenhouse gas emissions from the agriculture sector (Johnson and Johnson, 1995). Feed additives, such as tannins, can reduce emissions but often have broad-spectrum effects on the rumen microbes leading to negative effects on rumen fermentation (Patra *et al.* 2012). In the quest for natural origin, anti-methanogenic compounds (AMCs) that specifically target methanogens, we compared two plant-based AMCs (C, D-L) with three known synthetic AMCs (chloroform; nitroethane; monensin). We also tested whether supplements of metal ions (Ni; Fe; Co; Mn) that are cofactors of enzymes that catalyse methanogenesis would stimulate hyperactive methanogen groups.

Additives were tested using *in vitro* batch culture (Durmic *et al.* 2010) with oaten chaff as a fermentation substrate; the positive control (Cont) was oaten chaff only.

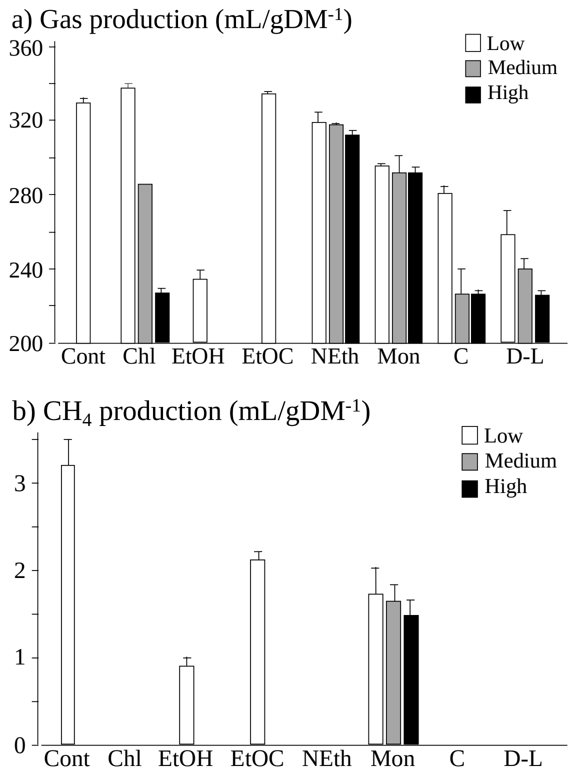

Figure 1. a) Total gas and b) methane production (mean ± standard error) i*n vitro* in the presence of methane inhibitors (C, D-L). Cont = Control; Chl = Chloroform; EtOC = Ethanol with Oaten Chaff substrate; NEth = Nitroethane; Mon = Monensin.

All treatments were introduced directly into the fermentation tubes, except for monensin that had to be dissolved in ethanol (EtOH), necessitating an ethanol control. All treatments were tested in triplicate, at low, medium and high doses. Doses of metal ions and synthetic AMC were selected on the basis of the literature (e.g., Hassanat and Benchaar 2013, for monensin). For C and D-L, doses were selected from preliminary testing in our laboratory. To assess the effects of treatments on fermentation, total gas, methane and hydrogen production were measured after 24 h incubation. Data were analysed by one-way ANOVA; means were compared with TUKEY's test.

Both C and D-L inhibited fermentation in a dose-responsive fashion, reducing total gas production by 15-30% compared to Cont (Fig 1a). Moreover, with C and D-L, methane was undetectable so the reduction was greater than with monensin ($P < 0.001$; Fig 1b). Metal ions did not increase methane production but increased total gas and hydrogen production ($P < 0.001$; Table 1).

Table 1. Effects of metal ion supplementation on rumen fermentation *in vitro*

Metal	Total gas (mL/gDM^{-1})			Methane (mL/gDM^{-1})			Hydrogen (mL/gDM^{-1})		
	L	M	H	L	M	H	L	M	H
Ni	315	321	314	0	0	0	24	25	25
Co	312	307	312	0	0	0	25	24	24
Fe	314	314	318	0	0	0	25	25	25
Mn	315	313	318	0	0	0	15	24	16

We conclude that both of the plant-based anti-methanogenic compounds are suitable candidates for a targeted approach to methane mitigation because they have only small adverse effects on rumen fermentation. The next step is to test the persistence of their effects over time. Our hypothesis that supplementation with metal ions would increase *in vitro* methane production was not supported but the increase in total gas and hydrogen production suggested that they aided fermentation, so further work is clearly needed.

References

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