

***Eremophila glabra* and *Kennedia prorepens* Reduce Methane Emission from *Medicago sativa* In Vitro**

X.X. Li^{AB}, Z. Durmic^{AB}, S.M.Liu^A, and P.E. Vercoe^{AB}

^ASchool of Animal Biology, The University of Western Australia, Crawley W.A. 6009

^BFuture Farm Industries CRC, The University of Western Australia, Crawley W.A. 6009

Common pasture species such as legumes provide good nutritive value for animals, but they can also be associated with high methane emissions from rumen fermentation. Australian plants contain bioactive secondary compounds with the capability to modulate gut microbes, for example, *Eremophila glabra* (EG) and *Kennedia prorepens* (KP) have been reported to modify rumen fermentation *in vitro* (Hutton, 2009). When these plants are used as the sole substrate and at a high dose rate *in vitro*, they have strong inhibitory effects on rumen fermentation, as judged by reduced gas, volatile fatty acid (VFA) and ammonia (NH₃) production. However, we have observed that when these plants are used at lower doses (i.e. 0.25 g/50 mL of incubation fluid), they have an antimethanogenic without negatively effecting overall rumen fermentation. We expect that these plants have the potential to reduce methane emissions when used as a plant mix rather than as a sole substrate. We tested this by measuring the *in vitro* fermentation characteristics of both plants when supplied as a sole substrate or in a mix with *Medicago sativa* (MS), a common pasture species.

A batch incubation *in vitro* system was used to compare the effects of *E. glabra* (EG) and *K. prorepens* (KP) on *in vitro* fermentation when used as the sole substrate or mixed with *M. sativa* (MS). The treatments included each plant as a sole substrate (0.5 g of EG, KP or MS), as well as two plant mixes: MS/EG (0.25 g MS, 0.25 g EG) and MS/KP (0.25 g MS, 0.25 g KP). Controls included sheep rumen fluid (no substrate, NS) and rumen fluid plus 0.5 g oatens chaff (standard reference, SR). The treatments were incubated with 50 mL buffered sheep rumen fluid in 100 mL crimped serum vials for 24 hours at 39° C. The cumulative gas pressure, concentrations of VFA, NH₃ and methane (CH₄), were measured and used as indicators of rumen fermentation (Table 1).

Table 1. The parameters of rumen fermentation *in vitro*

Parameters	NS	SR	MS	EG	KP	MS/EG	MS/KP
Gas (kPa)	36 ^f	119 ^b	121 ^{ab}	98 ^d	76 ^e	125 ^a	111 ^c
VFA (mmol/L)	70 ^c	115 ^a	125 ^a	99 ^b	89 ^b	116 ^a	116 ^a
NH ₃ (mg/L)	190 ^b	150 ^c	340 ^a	60 ^e	100 ^d	200 ^b	190 ^b
CH ₄ (ml/g)	12 ^d	32 ^c	70 ^a	31 ^c	36 ^c	55 ^b	54 ^b

Values with different superscripts in the same row differ significantly ($P < 0.05$)

When *E. glabra* and *K. prorepens* were included as the sole substrate in *in vitro* fermentations, they restricted fermentation compared to *M. sativa*, with significantly lower levels of gas, VFA and NH₃ produced, as well as a reduction in methane concentration around 50% (Table 1). When mixed with *M. sativa*, the inclusion of *E. glabra* did not affect gas. In contrast, addition of *K. prorepens* resulted in a lower gas production than *M. sativa* ($P < 0.05$). Both treatments where plant mixes were used as substrate reduced CH₄ and NH₃ concentrations ($P < 0.05$) but did not influence VFA production when compared to the *M. sativa* treatment. Gas production was not affected by the MS/EG treatment when compared to *M. sativa* treatment and was only slightly lower in the MS/KP treatment.

Our expectation that *E. glabra* and *K. prorepens* when used in a plant mix have the potential to retain their modulating effect on rumen methanogenesis while allowing normal fermentation is supported by our results. It is likely that bioactive compounds present in *E. glabra* and *K. prorepens* are responsible for this specific antimethanogenic effect and our further research will focus on isolating and identifying bioactive compounds from *E. glabra* and *K. prorepens* that reduce methanogenesis.

Hutton P., White C. L., Durmic Z., and Vercoe P. E. (2009). *Anim.* **3**, 1254.

Email: lix13@student.uwa.edu.au