Effect of legume and perennial ryegrass herbages on *in vitro* methane output using the total gas production technique

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Introduction In grass based beef production systems, the type and quality of herbage offered may have an influence on enteric methane (CH_4) emissions. Methane not only contributes to global warming (McGettigan *el al.*, 2008), it also represents an energy loss from the diet. The current study aims to compare the methane output from contrasting herbages at various stages of maturity.

Material and methods Herbage samples were obtained from a randomized complete block (n=4) designed field plot experiment in each of two successive years (Y). The herbage treatments (Hb) were *Lolium perenne* that received 0 kg [G1] or 150 kg [G2] of inorganic N fertiliser/ha/cut and two cultivars of *Trifolium pratense* (Merviot [L1] and Ruttinova [L2]). Independent plots of each herbage were harvested (Hv) twice during the primary growth (late May [I] & mid June [II]) and during an autumn regrowth [III]. Dried (40°C; 48h) milled (1mm) herbage samples were incubated in rumen fluid according to Mauricio *et al.* (1999). Methane and total VFA concentrations were determined using gas chromatography, for gas and liquid samples taken 24h after inoculation. Data were analysed using the PROC MIXED procedure in SAS considering observation from different years as repeated measures.

Results Chemical and fermentation characteristics of herbages are shown in Table 1. Crude protein (CP) content of the herbages was higher in the autumn (III) than in the early summer harvests (I & II) and negatively correlated (P<0.001) with water soluble carbohy-drates (WSC) and NDF content. Both Hb and Hv significantly influenced (P< 0.05) all variables displayed in Table 1. Red clovers (L1 & L2) had a lower CH_4 output per g DM incubated (P<0.05) compared to non-fertilised ryegrass (G1) and fertilised ryegrass (G2) had a lower output of CH_4 per g digested (P<0.05) than L1. Autumn harvest (III) had a lower output of CH_4 per g DM digested (P<0.05) than harvests I and II. There was a negative correlation (P<0.001) between CP content and CH_4 output (per g inc. and dig.).

Table 1 Herbage chemical composition, *in vitro* methane output and fermentation characteristics

Herbage (Hb)	Harvest (Hv)	CP *g/kg DM*	WSC[1] *g/kgDM*	NDF *g/kgDM*	aDMd[2] *g/g*	CH_4inc[3] *mmol/g DM*	CH_4dig[4] *mmol/g DM*	tVFA[5] *mmol/L*	C2:C3[6]
G1	I	98	236	475	0.65	1.82	2.78	53	2.85
	II	100	137	542	0.58	1.65	2.83	46	2.77
	III	186	131	474	0.67	1.66	2.50	48	3.02
G2	I	170	97	503	0.63	1.67	2.65	49	3.11
	II	163	41	563	0.54	1.52	2.84	44	3.01
	III	261	86	451	0.71	1.55	2.22	46	3.24
L1	I	175	65	404	0.54	1.64	3.05	45	3.64
	II	176	33	494	0.52	1.48	2.87	41	3.36
	III	284	56	341	0.63	1.50	2.45	43	3.40
L2	I	180	66	421	0.58	1.62	2.68	45	3.64
	II	186	53	439	0.49	1.50	3.06	42	3.46
	III	287	52	354	0.63	1.50	2.37	44	3.35
SEM (Hb x Hv x Y)[7]		7.3	12.8	15.1	0.026	0.034	0.111	1.7	0.084
Y		<0.001	<0.001	<0.001	<0.001	<0.001	0.329	<0.01	<0.001
Hb x Hv		0.309	<0.001	<0.01	0.155	0.569	0.064	0.534	<0.05
Hb x Y		0.063	<0.001	<0.05	0.522	0.285	0.587	0.087	<0.05
Hv x Y		<0.001	<0.01	<0.001	<0.001	<0.05	<.001	0.504	<0.001
Hb x Hv x Y		0.125	<0.001	0.052	<0.05	0.001	0.332	0.017	0.356

[1]WSC- Water soluble carbohydrates; [2] aDMd- apparent DM disappearance; [3]CH_4inc- methane expressed per g of DM incubated; [4]CH_4dig- methane expressed per g of DM digested; [5]tVFA-total fermentation VFA; [6]C2:C3- ratio acetate to propionate; [7]n = 4.

Conclusions In general, CH_4 output was lowest in herbages with higher CP content. Fertilised ryegrass had a lower CH_4 output per g DM digested than Merviot red clover. Autumn harvest showed a lower methanogenic potential than early summer harvests.

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