BRIEF COMMUNICATION: Evaluating rumen fluid from sheep and cattle as inoculum in a newly developed automated *in vitro* rumen batch culture system

S. MUETZEL*, C.L. HUNT and M.H. TAVENDALE

AgResearch Grasslands, Private Bag 11-008, Palmerston North 4442, New Zealand
*Corresponding author: stefan.muetzal@agresearch.co,.nz

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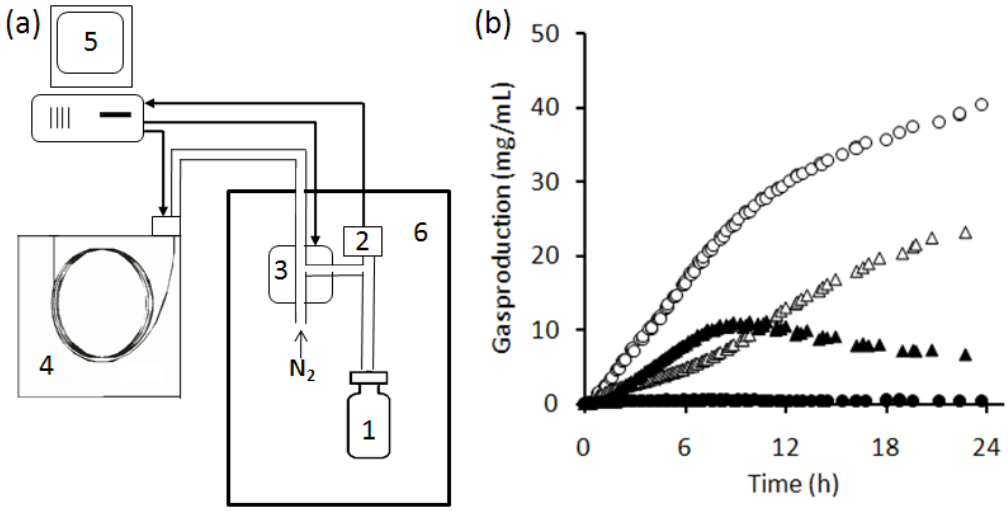
INTRODUCTION

In vitro rumen batch culture systems are widely used to rank feeds according to their rate of fermentation, potential gas production and *in vitro* digestibility. Their main advantage is that the analysis is quick and inexpensive. In these systems the gas can be either measured volumetrically in syringes (Menke *et al.*, 1979) or calculated from the pressure build up in bottles (van Gelder *et al*., 2005). Pressure based systems can and have been automated to obtain more information about the kinetics of gas production (van Gelder *et al*., 2005; Davies *et al*., 2000). The system presented here is a fully automated batch culture that in addition to measuring total gas production determines also the concentration of methane and hydrogen. In this experiment this *in vitro* system was used to study the effect sheep and cattle rumen fluid on total gas, methane and hydrogen production from various substrates.

MATERIALS AND METHODS

The system consists of 32 bottles, each connected to a pressure sensor and computer controlled three way solenoid valve. The pressure within each bottle was recorded every minute and when a bottle's pressure exceeded 9 kPa, the gas was released through a gas sample loop to the atmosphere. Immediately after venting, the gas sample loop was switched in line with a gas chromatograph equipped with a thermal conductivity and a flame ionisation detector for the measurement of hydrogen and methane respectively (Figure 1a). Calibration of produced gas volumes were carried out for each individual sensor by injecting into each bottle volumes of gas ranging from 1 to 10 mL. Calibration of the gas chromatography system was carried out by using gas alpha standards containing 2, 5, 10, and 20% methane and 1, 2.5, 5 and 10% hydrogen in nitrogen.

FIGURE 1: (a) Schematic description of the incubation system: 1. Incubation bottles; 2. Pressure sensor; 3. Solenoid valve; 4. Gas chromatograph; 5. Control PC; 6. Incubator; and (b): Methane (open symbols) and hydrogen (closed symbols) production profiles produced from quadruplicate bottles incubated with the negative (circles) and the positive control pasture hay (triangles).



Substrates (10 mg/mL) were incubated in 100 mL serum bottles containing 60 mL buffered rumen fluid comprising of 20% filtered rumen fluid and 80% buffer (Mould, *et al*., 2005).

All manipulations were performed at 39°C under a stream of CO_2. The following substrates were used to test the effect of the source of the inoculum; wheat straw, concentrate (60% wheat, 40% lucerne), lucerne (pelleted), chicory, ryegrass, white clover. The crude protein content of the substrates was 35, 176, 223, 197, 212 and 288 g/kg dry matter (DM) and neutral detergent fibre content was 806, 240, 451, 282, 526 and 361 g/kg DM respectively. The substrates were freeze dried where applicable and ground to pass a 1mm sieve in a Wiley mill and were incubated with rumen fluid from either sheep or cattle. Substrates were incubated for 48 hours in duplicate bottles using rumen fluid from one donor animal only. The incubation was repeated with rumen fluid from four sheep and cattle (n = 4). The rumen fluid donor animals were all fed with pasture hay at maintenance twice a day (08:00 and 16:00 h) in equal proportions and rumen fluid was collected before the morning feeding. Each incubation run contained a negative (pasture hay) and a positive (pasture hay plus 30 μM bromoethane-sulphonate, BES) control to assess the activity of the incubation and the effectiveness of a methane inhibitor respectively.

RESULTS

Over a 24 hour period between 15 and 25 gas samples were analysed per bottle. The repeatability of the gas composition results is demonstrated in Figure 1(b), where four bottles containing the positive and negative control were incubated showing that inhibition of methanogens with BES leads to a release of hydrogen into the headspace. When substrates were incubated with rumen fluid from four animals the system was able to resolve a difference in gas and methane production of 7.0 and 13.4% respectively with a power of 95% at a level of $\alpha < 0.05$.

Gas production was similar when substrates were incubated with rumen fluid from sheep and cattle, but the proportion of methane and hydrogen was lower when rumen fluid from sheep was used (Table 1). A significant interaction between animal species and substrate was only observed for hydrogen.

Total gas production was different for the substrates incubated, with pasture hay and wheat straw leading to the lowest and the concentrate to the highest gas production. The proportion of methane was highest for the wheat straw and ryegrass hay and lowest for chicory while the concentrate ranked in the middle, but showing the highest release of hydrogen.

TABLE 1: Total gas production and proportion of methane and hydrogen from seven substrates incubated with rumen fluid from two different donor species. SED = Standard error of the difference. Bolding of P values indicates significance (P <0.05).

Comparison	Treatment	Gas production (mL/g)	Methane (%)	Hydrogen (%)
Substrates	Chicory	156.3	13.8	0.22
	Pasture hay	127.6	16.3	0.20
	Lucerne	136.9	15.3	0.17
	Ryegrass	167.7	15.2	0.34
	Wheat straw	128.2	16.5	0.18
	Concentrate	199.9	15.5	0.77
	White clover	171.7	14.6	0.44
	SED	5.7	0.5	0.11
	P value	**<0.001**	**<0.001**	**<0.001**
Donor species	Cow	150.2	15.0	0.43
	Sheep	151.3	13.6	0.24
	SED	3.1	0.3	0.06
	P value	0.51	**0.006**	**0.005**
Interaction	P value	0.59	0.55	**<0.001**

DISCUSSION

In the past several automated rumen *in vitro* incubation systems have been developed (Beuvink *et al*., 1992; Cone *et al*., 1996; Davies *et al*., 2000), however in contrast to the system presented here, these systems do not measure gas composition. Our system was developed as part of a strategy to identify potential methane mitigation agents by testing substrates or additives *in vitro* prior to *in vivo* studies. In this experiment the system was used to assess the difference in gas composition when rumen fluid from sheep and cattle was used. The gas production after 24 hours of incubation was similar for cattle and sheep, a

result also found by other researchers (Bueno *et al*., 2005; Cone *et al*., 2002) when donor animals are kept under similar conditions and fed the same diet. However our results show that cattle on average produced 4.2% more methane and more than 40% more hydrogen from the same substrates than sheep. Lower methane emissions in sheep compared to cattle have also been observed *in vivo* (Swainson *et al*., 2008a; Machmueller & Clark, 2006). However there was an interaction between substrate and animal species for the hydrogen results. Overall the hydrogen concentration was too low to account of the difference in methane observed. Not surprisingly the substrates used lead to differences in gas and methane production. Highly digestible substrates like the concentrate and white clover produced high amounts of gas while more fibre-rich substrates like the straw and pasture hay control sample resulted in low yields of gas production after 24 hours of incubation. The proportion of methane showed the opposite trends with high yields for fibre-rich substrates and comparatively low yields for substrates low in plant cell wall. The lowest proportion of methane *in vitro* was found for chicory. The results agree with *in vivo* studies where lower methane yield from chicory compared to ryegrass in sheep has been reported (Swainson *et al*., 2008b; Sun *et al.* 2010).

In conclusion the newly developed incubation system is a useful tool to study differences in gas composition from different ruminants and substrates and has the potential to identify plant based methane inhibitors.

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