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In vitro fermentation characteristics of diets with different forage/concentrate ratios: comparison of rumen and faecal inocula

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Abstract

BACKGROUND: The aim of this trial was to evaluate the replacement of rumen fluid with faeces as inoculum in studying the *in vitro* fermentation characteristics of diets for ruminants using the *in vitro* gas production technique. Six iso-protein diets with different forage/concentrate ratios were incubated with rumen fluid (RI) or faeces (FI) collected from sheep.

RESULTS: Most of the fermentation parameters were influenced by diet and inoculum ($P < 0.01$). With both inocula, organic matter degradability (dOM), cumulative gas production (OMCV) and maximum fermentation rate (R_{max}) increased as the amount of concentrate in the diet increased. R_{max} was lower with FI *vs* RI ($P < 0.01$); dOM was higher with FI *vs* RI and the diet × inoculum interaction was significant. As expected, with both inocula, R_{max} increased as the neutral detergent fibre content of the diet decreased. Significant correlations were obtained using both inocula between OMCV/dOM and gas/volatile fatty acid (VFA), while the correlation VFA/dOM was significant only with FI. The microbial biomass yield calculated by stoichiometric analysis for all diets was higher with FI *vs* RI. With FI the organic matter used for microbial growth showed an overall decreasing trend as the amount of concentrate in the diet increased.

CONCLUSION: The results indicate that both faeces and rumen fluid from sheep have the potential to be used as inoculum for the *in vitro* gas production technique.

Keywords: *in vitro* gas production; rumen fluid; faeces; diets; forage/concentrate ratio; stoichiometric calculations

INTRODUCTION

Digestibility is an important measure for evaluating ruminant feeds. Although it can be determined *in vivo*, this method is laborious and requires a relatively large number of animals. Biological digestion techniques used to determine the nutritive value of ruminant feeds include (1) *in vitro* incubation and digestion with rumen micro-organisms (e.g. Tilley and Terry,[1] the gas production method,[2,3] determination of true organic matter digestibility[4]) and (2) enzymatic degradation with cell-free fungal cellulase.[5] More recently, the interest in efficient utilisation of roughage diets has led to an increase in the use of the *in vitro* gas production technique owing to the advantages of studying the fermentation kinetics of both soluble and insoluble fractions of feeds.[6–8]

When a feed is incubated *in vitro* with buffered rumen fluid, the carbohydrates are fermented to produce volatile fatty acids, gases and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate. Gas production from protein fermentation is relatively small compared with carbohydrate fermentation.[9] The fermentation kinetics of feedstuffs can be determined from fermentative gas and the gas released from buffering of volatile fatty acids. The kinetics of gas production is dependent on the relative proportion of soluble, insoluble but degradable, and undegradable particles of the feed.

The mathematical description of gas production profiles allows analysis of the data as well as evaluation of substrate differences and the fermentability of soluble and slowly fermentable components of feeds. Several gas-measuring techniques and *in vitro* gas methods are in use by several groups,[3,10–12] and all highlight the disadvantage of requiring fresh inocula from permanently fistulated animals. Hence the use of alternative inocula has been proposed: bovine rumen fluid from a slaughterhouse[13,14] and faecal micro-organisms contained in a filtered suspension of ruminant faeces.[15–19] Using alternative sources of inocula makes a significant contribution to animal welfare. In addition, it is much easier to obtain faeces or rumen fluid from a slaughterhouse than to obtain rumen fluid from a fistulated animal. El-Meadaway *et al*.[20] indicated that bovine faecal inoculum has the potential to be used instead of bovine rumen inoculum to obtain *in vitro* dry matter digestibility and gas production for barley grain, Persian clover, alfalfa and bromegrass forage hay, but not for poor-quality

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roughages such as barley straw. Fermentation with faeces has a longer lag phase, a slower rate of gas production and lower volatile fatty acid production than fermentation with rumen fluid.

Jones and Barnes,[21] using the Tilley and Terry[1] technique, reported that the *in vitro* digestibility values of shrub legumes obtained with faecal fluid were lower than those obtained with rumen fluid, albeit linearly correlated ($r = 0.892$, $P < 0.001$). Also, Mauricio *et al*.[22] concluded that, with forages of contrasting digestibility, faecal matter has potential as an alternative inoculum to rumen liquor for the *in vitro* gas production technique, although with faeces-based inoculum a consistently longer lag phase was observed. However, the results in the literature are not in total agreement, and the hope is widely expressed that further studies will improve the use and results of faeces as inoculum. As all the above authors used only a single feedstuff as substrate for incubation, the aim of this trial was to evaluate the replacement of rumen fluid with faeces as inoculum in studying the *in vitro* fermentation characteristics of six diets with different forage/concentrate ratios for ruminants using the *in vitro* gas production technique.

MATERIALS AND METHODS

Rumen inoculum (RI)

Rumen fluid was obtained before morning feeding from five dry crossbreed (Delle Langhe × Comisana) sheep (~50 kg live weight) fed for 3 months on oat hay *ad libitum*. The rumen fluid, transferred to the laboratory in a warmed thermos flask (39 ± 0.5 °C), was squeezed through four layers of gauze and combined among sheep. The retained solids were then mixed with a volume of anaerobic buffer (medium D[23]) equal to the volume of rumen fluid obtained. The suspension was homogenised in a blender for 60 s and the homogenate was strained through two layers of gauze. The resulting fluid was combined with the other strained fluid and held at 39 °C. The various steps were carried out under a constant stream of CO_2 to maintain anaerobic conditions.

Faecal inoculum (FI)

Faeces were collected directly from the rectum immediately after sampling of rumen fluid from the same animals. Faeces samples were placed in a prewarmed thermos flask. The fresh faeces from all sheep were combined and stirred with a glass pestle, then a 50 g sample was diluted with 100 mL of anaerobic buffer.[23] The resulting suspension was strained through two layers of gauze. The remaining solids were then resuspended in 100 mL of anaerobic buffer and homogenised by blending for 60 s. The homogenate was strained through two layers of gauze, mixed with the first strained solution and held at 39 °C. The various steps were carried out under a constant stream of CO_2 to maintain anaerobic conditions.

Substrates

The substrates were six iso-protein diets (N × 6.25 = 115 g kg^{-1} dry matter) with different forage/concentrate ratios: diet 1, 100/0 (all hay); diet 2, 90/10; diet 3, 80/20; diet 4, 70/30; diet 5, 60/40; diet 6, 50/50. For all diets, oat (*Avena sativa* L.) hay was used as forage and the concentrate consisted of corn meal (64%) and wheat bran (36%). Dry matter (DM), ash, crude protein (CP) and ether extract (EE) contents were determined according to AOAC[24] methods 930.04, 930.05, 977.02 and 920.39 respectively. Neutral detergent fibre (NDF) was determined as proposed by Van Soest *et al*.[25] Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest.[4]

In vitro trial

Samples (~1 g) of each diet, ground to pass a 1 mm screen (Braebender Wiley mill, Braebender OHG, Duisburg, Germany), were incubated in triplicate with both inocula at 39 °C in 120 mL culture flasks containing 74 mL of anaerobic medium (modified medium D[23]), 5 mL of reducing agent and 10 mL of inoculum.[26] The medium does not supply nitrogen. Fermentation was carried out for 144 h. At pre-established times (at 2–24 h intervals) the gas produced was measured for each flask using a manual system consisting of a pressure transducer described by Theodorou.[23] At the end of incubation, 5 mL of the liquid in each flask was used to determine pH (Alessandrini Instrument glass electrode, Jenway 3030, Jenway, Dunmow, UK) and volatile fatty acids (VFAs). The content of each flask was filtered using preweighed crucibles (porosity 2; Schott Duran, Mainz, Germany), and residual organic matter (OM) was determined by drying overnight at 103 °C and burning at 550 °C for 4 h. Degraded OM (dOM, %) was calculated as the difference between incubated and residual OM, corrected for the blank, which consisted of four flasks containing only buffered inoculum.

For VFA analysis the liquid sample was centrifuged twice at 12 000 × *g* for 10 min at 4 °C, then 1 mL of supernatant was added to 1 mL of 0.06 mol L^{-1} oxalic acid. VFAs were measured by gas chromatography (Thermo Quest GC 8000TOP, ThermoQuest Italia, SpA, Rodano, Milan, Italy) using a fused silica capillary column (30 m × 0.25 mm, 0.25 mm film thickness). Acetate, propionate, butyrate, isobutyrate, valerate and isovalerate were used as external standards. The area of each VFA response was compared with those of the external standards.

Stoichiometric calculations

Theoretical gas production and OM fermentation were estimated according to Groot *et al*.[27] and based on the stoichiometric balance equations of Van Soest,[28] by use of the VFAs measured at the end of fermentation. OM fermentation was expressed in glucose equivalents (g). It was assumed that the glucose equivalents were fermented to form acetic (HAc), propionic (HPr) and butyric (HBu) acids and the gases CO_2 and CH_4, as well as being incorporated into microbial biomass. From stoichiometric equations it can be calculated that

$$CO_2 = HAc/2 + HPr/4 + 3HBu/2$$
$$CH_4 = HAc + 2HBu - CO_2$$

Glucose consumption for production of products (OM fermented) was calculated as

$$\text{OM fermented} = 162(2HAc + 3HPr + 4HBu + CO_2 + CH_4)/6$$

The amount of OM utilised for microbial synthesis (Y_M) was estimated[25] as the difference between degraded (measured) OM (Y_D) and fermented (predicted) OM (Y_F). These equations were also used with faecal inoculum by Váradyová *et al*.[19]

Calculations and statistical analysis

For both inocula (RI and FI) the cumulative gas volumes for each bottle obtained at each incubation time, reported as mL g^{-1}

Table 1. Ingredients and chemical composition of diets

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredients (g kg^{-1} as fed)						
Oat hay	1000	900	800	700	600	500
Concentrate[a]	0	100	200	300	400	500
Chemical composition (g kg^{-1} DM)						
CP	115.1	117.2	116.2	116.2	113.2	114.2
EE	20.0	22.3	23.6	26.3	27.7	29.2
NDF	639.3	596.4	560.4	525.1	479.2	444.5
ADF	436.2	405.1	360.2	312.2	282.1	240.7
ADL	73.1	68.0	62.0	56.0	50.0	43.0
NFC	129.4	176.1	215.8	260.4	311.9	330.1
Ash	96.2	88.0	84.0	72.0	68.0	82.0

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; NFC = 100 − (NDF + CP + ash + EE), non-fibre carbohydrates.
[a] Consisting of 64% maize meal and 36% wheat bran.

incubated OM, were fitted to the model[29]

$$G = A/(1 + B/\text{time})^C$$

where G (mL g^{-1}) is the total gas produced, A (mL g^{-1}) is the asymptotic gas production, B (h) is the time at which $A/2$ is reached and C is the switching characteristic of the curve. The maximum gas production rate (R_{max}, mL h^{-1}) and the time at which it occurs (T_{max}, h) were also calculated according to the formulae[30]

$$R_{max}(\text{mL h}^{-1}) = [(A \times C^B) \times B \times (T_{max}^{-B-1})]/[(1 + C^B) \times (T_{max}^{-B})^2]$$
$$T_{max}(\text{h}) = C \times [(B - 1)/(B + 1)]^{1/B}$$

The *in vitro* fermentation characteristics such as dOM, OMCV (mL cumulative gas production at 144 h g^{-1} incubated OM), yield (mL cumulative gas production g^{-1} disappeared OM), VFA (mmol l^{-1} g^{-1} incubated OM), pH and the fitted parameters were subjected to analysis of variance (PROC GLM of SAS[31]) in order to detect the effects of inoculum (RI and FI) and substrate (diets 1–6). The substrate × inoculum interaction was also included in the model. The correlations between the fermentation parameters obtained with rumen fluid and faeces were also studied using PROC REG of SAS.[31] Significant differences between treatment means at the 1% significance level were evaluated using Tukey's multiple range test.

RESULTS AND DISCUSSION

The ingredients and chemical composition of the diets are reported in Table 1. The CP content of the diets was intentionally low in order to avoid interactions during the *in vitro* fermentation process. However, according to MacDonald *et al.*,[32] the substrate protein ensured that the maintenance requirement for microbial protein synthesis was satisfied.

Gas and fermentation rate profile

The cumulative gas production and fermentation rate over time for each diet with both inocula are reported in Fig. 1. The chosen model fits the data of gas production obtained satisfactorily (r^2 from 0.9843 to 0.9996 and standard error of estimate from 1.64 to 8.22). Gas production was always lower with FI *vs* RI. The reason for this trend may be attributed both to the different nature of the micro-organisms in the faeces with respect to the rumen fluid[22,33] and to the 'survival state' of faecal bacteria with low metabolic activity.[22]

With RI the rate of gas production was higher than with FI and showed the classical parabolic trend: it increased up to a maximum, decreased rapidly until 48 h of incubation and then slowly tended to zero. The FI behaviour was more complex: diets 1 and 6 showed parabolic trends, while diets 2, 3 and 5 formed an almost incomplete parabola on the left.

In general, with FI the rate decreased more slowly and the curve seemed flatter, testifying to a less intense fermentation process. As expected for both inocula, R_{max} tended to increase as the NDF content of the diet decreased ($R^2 = 0.960$, $P < 0.01$ and $R^2 = 0.845$, $P < 0.05$ for RI and FI respectively).

Degraded organic matter, rates and extent of gas production, VFA production

The parameters of *in vitro* fermentation (dOM, OMCV, yield, A, B, R_{max} and T_{max}) of the six diets with the two inocula are shown in Table 2. All parameters were affected ($P < 0.01$) by diet and, except for A and T_{max}, also by inoculum. There was a significant diet × inoculum interaction for all fermentation characteristics except R_{max}. For both inocula, OM degradability and OMCV values increased as the amount of concentrate in the diet increased (Fig. 2). In particular, with RI the first three diets presented lower dOM values ($P < 0.01$) than the other three diets richer in concentrate. Concerning OMCV, a significant effect of concentrate was evidenced only for the diets with 40 and 50% concentrate (diets 5 and 6, $P < 0.01$) compared with the other diets. With FI the hay diet (diet 1) showed a significantly ($P < 0.01$) lower dOM value than the other five diets, while for the concentrate-rich diet (diet 6) this parameter was significantly higher only compared with diets 1, 2 and 5. For OMCV the trend was more linear: diet 1 was the lowest ($P < 0.01$) while diet 6 was the highest ($P < 0.01$). It was not possible to compare these results with previous findings, since the incubation of diets differing in F/C ratio has not been attempted elsewhere. However, concentrate usually shows higher OM degradability and gas production compared with grass.[19] With RI, yield (gas cumulative volume at 144 h g^{-1} OM disappeared) and asymptotic gas production (A, mL g^{-1}) increased from diet 1 to diet 6 (from 382 to 418 mL g^{-1} and from 259 to 316 mL g^{-1} for yield and A respectively). These two parameters showed an irregular trend with FI.

Comparing the two inocula, OM degradability was higher with FI *vs* RI (dOM 77.15 *vs* 74.54%), while OMCV (253 *vs* 292 mL g^{-1}), yield (326 *vs* 391 mL g^{-1}) and A (275 *vs* 283 mL g^{-1}) were lower with FI *vs* RI. Figure 2 shows that the difference in OM degradability between the inocula was significant only for diet 2 ($P < 0.01$) and diet 3 ($P < 0.001$), while for OMCV the differences between RI and FI were significant in all diets except diet 4.

As regards gas production, it is widely reported[19,22,34] that faeces produce less gas than rumen fluid. This is probably due to the fact that faecal micro-organisms originate mainly in the caecum/colon, where fermentation activity is lower than in the rumen. According to Mauricio *et al.*,[22] lower fermentation (i.e. less gas production) in the caecum/colon is the result of several factors, such as provision of substrates to the rectum with lower nutritive value, shorter retention times, lower populations of bacteria or absence of protozoa, compared with the rumen. Regarding the

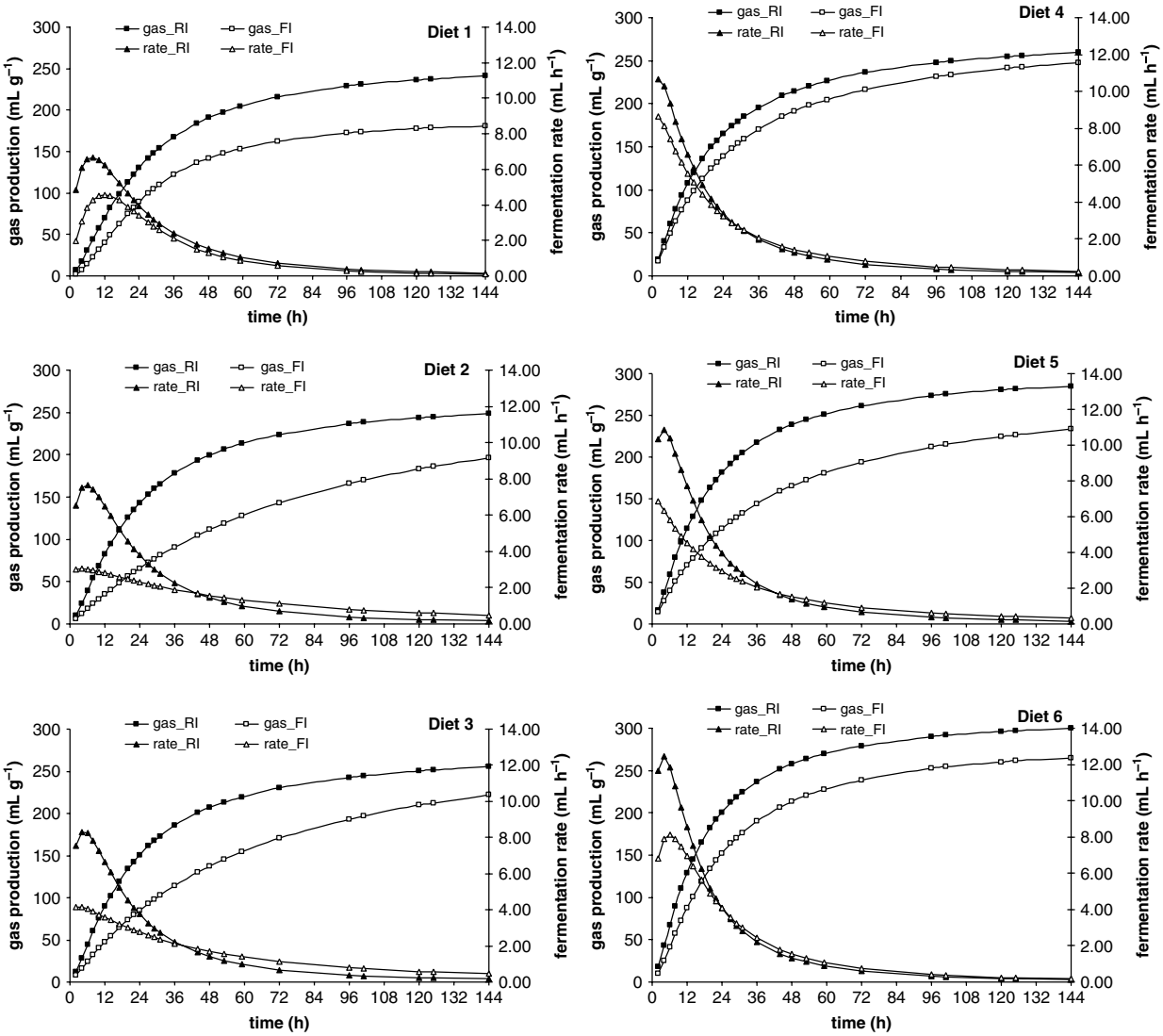

Figure 1. *In vitro* gas production and fermentation rate over time for six diets incubated with rumen (RI) and faecal (FI) inocula.

maximum rate of gas production, other authors[22,35] also reported higher values with rumen fluid than with faeces and, with both inocula, a reduction in this parameter with increasing NDF content of the substrate.[19]

With both inocula the maximum gas production rate increased as the amount of concentrate in the diet increased. In particular, with RI and FI, diets 1 and 2 presented significantly ($P < 0.01$) lower R_{max} compared with diets 4, 5 and 6 (Table 2), testifying to the higher energy available for microbial populations supplied by the concentrate. With RI, *B* values occurred earlier from diet 6 to diet 1 (from 15.9 to 23.7 h), while with FI the trend was irregular. Comparing the two inocula, *B* values occurred later with FI *vs* RI (40.40 *vs* 19.55 h) and R_{max} was lower with FI *vs* RI (5.98 *vs* 9.51 mL h^{-1}). Figure 2 shows that R_{max} was always higher with RI and that the differences were statistically significant ($P < 0.01$) except for diet 4.

VFA production and pH values of the six diets with the two inocula are shown in Table 3. Total VFA and acetate production were numerically lower with FI *vs* RI, though the differences were not statistically significant; the diet × inoculum interaction was not significant. pH values, lower with RI *vs* FI (6.55 *vs* 6.59, $P < 0.01$), followed the trend of total VFA values. The acetate/propionate (A/P) ratio was higher with RI *vs* FI (2.24 *vs* 2.16) in accordance with the higher gas production, though the differences between the two inocula were not statistically different.

The use of faecal material as an alternative to rumen fluid has been extensively explored by the Tilley and Terry[1] technique. El-Meadaway *et al*.[20] and Butler *et al*.[36] reported that faecal estimates of OM digestibility were overall lower than those obtained using rumen fluid, but the effect of inoculum source was not significant. Others[21,37] showed that OM digestibility determined with an inoculum prepared with faeces was significantly lower than that obtained with an inoculum prepared with rumen fluid. Van der Baan *et al*.[38] found no differences in the *in vitro* OM digestibility of *Atriplex nummularia*, supplemented with different levels of maize and barley, using RI or FI. Borba and Ramalho Ribeiro[18] studied the *in vitro* OM digestibility of grass forages and found higher values with FI *vs* RI, the difference being significant ($P < 0.05$) for perennial ryegrass.

Others have used a liquid suspension of faeces as inoculum in the *in vitro* gas production technique. Cone *et al*.[12] concluded that faeces from cows can be used as an alternative to rumen

Table 2. *In vitro* fermentation characteristics of diets differing in forage/concentrate (F/C) ratio and incubated with rumen (RI) and faecal (FI) inocula

Inoculum	Diet	dOM (%)	OMCV (mL g^{-1})	Yield (mL g^{-1})	*A* (mL g^{-1})	*B* (h)	T_{max} (h)	R_{max} (mL h^{-1})
RI	1	69.45C	265.3B	382.2BC	258.6B	23.7	7.49A	6.74C
	2	71.64C	270.4B	377.6BC	267.5B	21.7	5.45AB	7.85C
	3	71.87C	279.3B	392.0ABC	274.8B	20.7	4.70AB	8.48BC
	4	76.58B	284.5B	371.6C	281.5AB	17.5	2.20B	10.51AB
	5	78.26AB	316.5A	404.6AB	303.8A	17.8	3.70B	10.95A
	6	79.96A	333.8A	417.6A	316.0A	15.9	3.80B	12.52A
FI	1	69.41C	212.0D	307.4CD	190.0B	25.6CD	11.83A	4.25C
	2	75.49B	222.7C	286.8D	294.7A	75.7A	4.01BC	3.16C
	3	78.61AB	239.8C	305.1D	304.7A	57.6B	2.84BC	4.38BC
	4	79.07AB	267.4B	338.4BC	284.0A	24.9CD	1.56C	8.67A
	5	78.17B	270.1B	345.4AB	292.0A	37.0C	0.40C	7.19AB
	6	82.15A	306.3A	373.0A	284.7A	21.7D	5.74B	8.23A
Diet (D)								
Probability		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MSD[a]		2.69	15.80	23.10	26.09	8.69	2.78	2.07
MSD[b]		3.27	19.30	28.11	31.87	10.61	3.41	2.54
Inoculum (I)								
Probability		<0.001	<0.001	<0.001	0.0971	<0.001	0.8032	<0.001
MSD[a]		1.05	6.21	9.04	10.28	3.42	1.09	0.82
MSD[b]		1.41	8.37	12.17	13.87	4.62	1.49	1.11
Interaction (D × I)		0.0021	0.0143	0.0031	<0.001	<0.001	0.0028	0.1675

For each inoculum, means with different letters within a column are significantly different at $P < 0.01$.
Diet 1, F/C = 100/0 (all hay); diet 2, F/C = 90/10; diet 3, F/C = 80/20; diet 4, F/C = 70/30; diet 5, F/C = 60/40; diet 6, F/C = 50/50; dOM, organic matter degradability (% of incubated); OMCV, gas cumulative volume related to incubated OM; yield, gas cumulative volume related to degraded OM; *A*, asymptotic gas production; *B*, time at which *A*/2 is reached; R_{max}, maximum rate of gas production; T_{max}, time at which maximum rate occurs.
[a] Minimum significant difference at 5%.
[b] Minimum significant difference at 1%.

fluid to accurately determine differences in total gas production but not to correctly estimate the fermentation rate. According to Mould *et al.*[17] and Rymer *et al.*,[39] faeces can replace rumen fluid as inoculum for end-point measures (i.e. degradability or cumulative gas volume at the end of incubation periods), but they are unsuitable for the estimation of fermentation rate. Our results show that *in vitro* OM degradability was higher with faeces than with rumen fluid, though the difference was significant only for diets 2 and 3. In contrast, Váradyová *et al.*[19] found higher DM digestibility with faeces than with rumen fluid, but the difference was undoubtedly affected by the different amounts of rumen fluid used: 5 mL g^{-1} substrate (in our case) *vs* 40 mL g^{-1} substrate. A wide range of inoculum concentrations have been used in the various gas production techniques. Raising the proportion of rumen fluid in the inoculum increased the volume of gas produced[40] and the maximum rate of gas production[10,41] and reduced the lag time.[10]

Comparison between predicted and observed gas volume

The differences between predicted and observed values of gas production are summarised in Fig. 3. Gas production estimated using the stoichiometric equations of Van Soest *et al.*[25] takes into account the direct gas from VFAs (from fermentation) and the indirect gas released from the bicarbonate buffer. For RI the predicted values were higher than those measured for diets 1 and 3, lower for diet 4 and similar for diets 2, 5 and 6. For FI the stoichiometrically predicted gas volume was higher than the measured gas volume for all diets except diet 1. Predicted and measured gas volumes were correlated with both inocula ($R^2 = 0.839$, $P < 0.05$ for RI, $R^2 = 0.753$, $P < 0.05$ for FI and $R^2 = 0.806$, $P < 0.0001$ for RI + FI; data not reported).

The calculated volume of gas comprises three components: CO_2 and CH_4 from fermentation and CO_2 released from HCO_3 upon buffering the VFAs generated. After 144 h of fermentation the average proportions with RI were 0.242 CO_2 and 0.144 CH_4 from fermentation and 0.614 CO_2 from buffer. These values were almost identical to those obtained for faeces (0.244, 0.166 and 0.617 respectively for CO_2 and CH_4 from fermentation and CO_2 from buffer) and close to the values reported by Blümmel and Ørskov,[11] testifying that the fermentation process was qualitatively similar between inocula.

Relationship between estimates obtained by different inocula

Correlation coefficients of the *in vitro* fermentation data obtained with faeces or rumen fluid as inoculum are reported in Table 4. Total gas production for both inocula was related to dOM ($R^2 = 0.764$, $P < 0.05$ for faeces and $R^2 = 0.859$, $P < 0.01$ for rumen fluid). VFA production was related to dOM only for FI ($R^2 = 0.894$, $P < 0.01$); the high VFA variability probably influenced the lack of correlation between VFA and dOM for RI. There was a significant linear relationship between gas and VFA production for both inocula ($R^2 = 0.798$, $P < 0.05$ for FI and $R^2 = 0.859$, $P < 0.01$ for RI). The regression coefficient (37.6 mL gas mmol l^{-1} g^{-1} VFA) obtained for RI was consistent with that reported by Doane *et al.*[42] and Calabrò *et al.*[43] Gas and VFA production, degraded OM and fermentation rate of the six diets differed between the inocula, but

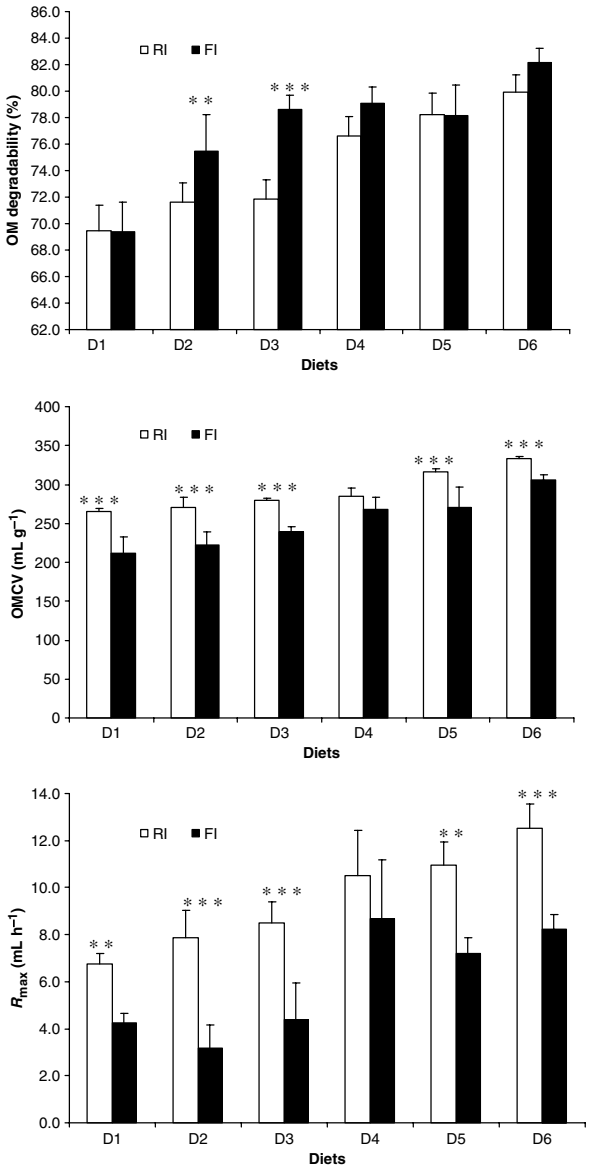

Figure 2. Comparison of rumen (RI) and faecal (FI) inocula for organic matter (OM) degradability, cumulative gas production related to incubated OM at 144 h (OMCV) and maximum rate of gas production (R_{max}) in diets differing in forage/concentrate (F/C) ratio: D1, F/C = 100/0 (all hay); D2, F/C = 90/10; D3, F/C = 80/20; D4, F/C = 70/30; D5, F/C = 60/40; D6, F/C = 50/50. $^{**}P < 0.01$; $^{***}P < 0.001$.

the data were correlated ($R^2 = 0.724$, $P < 0.05$ for gas production, $R^2 = 0.672$, $P < 0.05$ for VFA production, $R^2 = 0.867$, $P < 0.01$ for dOM and $R^2 = 0.878$, $P < 0.05$ for fermentation rate).

Váradyová *et al*.[19] studied the fermentation patterns of feeds using rumen fluid and fresh faeces as sources of inocula. The authors reported lower gas production, DM digestibility and total VFA values with rumen fluid than with fresh faeces; in addition, the data from these two inocula were generally poorly related. They concluded that FI cannot be used to replace RI. Our results agree substantially with findings elsewhere:[17,21,35,44–48] a close relationship was observed between FI and RI for end-point measures such as gas production and digestibility, despite the variability found by other authors.[19,20] Moreover, in our case the kinetic parameters were also well correlated between the inocula.

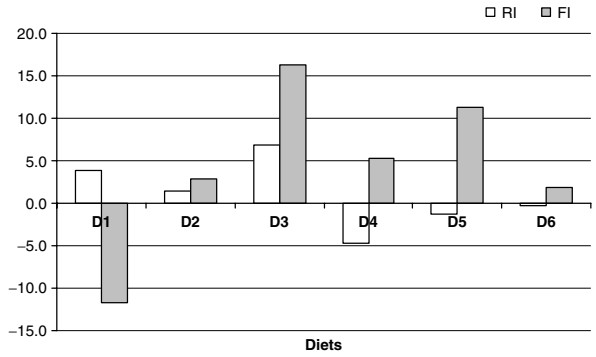

Figure 3. Differences between calculated and measured gas production (as % measured value) for diets differing in forage/concentrate (F/C) ratio: D1, F/C = 100/0 (all hay); D2, F/C = 90/10; D3, F/C = 80/20; D4, F/C = 70/30; D5, F/C = 60/40; D6, F/C = 50/50. RI, rumen inoculum; FI, faecal inoculum.

A contributory cause of these better results was the fact that the donor animals were fed oat hay for at least 3 months and the incubation period was quite long (144 h).[17] Akhter *et al*.[37] found that the *in vitro* OM digestibility of six forages determined using FI produced by donor animals fed hay was higher than that obtained using FI produced by donor animals fed concentrates or a mixture of hay plus concentrates. Moreover, the regression equations relating the *in vitro* OM digestibility of forages determined using RI from sheep and FI from cattle showed lower relative standard deviation values with FI from cattle fed hay than with FI from cattle fed hay plus concentrate. The authors concluded that the type of diet not only affects the activity of the FI but may also influence the error in predicting OM digestibility.

Many investigators have found that the activity of RI is affected by the diet of the donor. An appropriate diet will provide such nutrients to the hind gut while at the same time increasing the residence time of the feedstuff. Both factors should increase the numerical presence of cellulolytic bacteria, which explains the higher *in vitro* digestibility values obtained when diets for donor animals included hay or straw rather than grass.[15] Mauricio *et al*.[49] reported that the gas production parameters estimated in Brazil, where donor cows were fed a diet containing poor hay and little concentrate, were generally similar for RI and FI. This contrasts with the higher values for rumen liquor compared with faeces estimated previously in the UK, where the diet of the donor cows involved high-quality grass silage and a higher proportion of concentrate.

Organic matter used by microbial population

Figure 4 shows the OM fermented (Y_F) and that used for microbial biomass yield (Y_M) for each diet with RI and FI. For both inocula, Y_F estimated by VFA concentration was always lower than Y_D. This could be related to the microbial growth during *in vitro* incubation, which requires both nitrogen and carbon sources. For all diets the predicted amount of OM used for microbial growth (Y_M) was higher for FI than for RI, suggesting that a higher proportion of degraded OM was used for microbial biomass production in faeces. The diet of the donor animals, consisting only of forage, probably contributed to this result. Also, Van Vliet *et al*.[50] found that faeces obtained from cows fed a diet with mature grass silage showed a lower microbial biomass with higher activity (expressed as incorporation of ^{14}C-Leu) compared with faeces produced by cows fed a high-energy, high-protein diet.

Table 3. pH and volatile fatty acids (mmol l^{-1} g^{-1}) of diets incubated with rumen (RI) and faecal (FI) inocula

Inoculum	Diet	pH	tVFA	Acetate	Propionate	Butyrate	A/P
RI	1	6.56A	72.71	45.47	17.87	1.56	2.84
	2	6.61A	73.57	44.95	20.40	1.05	2.20
	3	6.58A	80.80	48.92	20.73	1.11	2.35
	4	6.60A	73.30	43.19	21.05	1.62	2.10
	5	6.48B	84.67	50.00	24.27	1.20	2.05
	6	6.45B	91.58	51.71	27.96	1.43	1.88
FI	1	6.60ABC	50.21	30.94	14.25B	0.49	2.38
	2	6.65A	58.91	36.69	16.09AB	0.50	2.72
	3	6.63AB	78.03	42.98	28.25A	0.70	1.55
	4	6.61AB	74.68	45.33	19.38AB	1.28	2.43
	5	6.54BC	81.36	49.63	23.12AB	1.49	2.26
	6	6.51C	86.54	47.77	29.86A	1.27	1.62
Diet (D)							
Probability		<0.001	0.090	0.343	0.0105	0.4268	0.3512
MSD[a]		0.071	29.30	18.30	9.570	1.07	1.13
MSD[b]		0.087	36.00	22.40	11.70	1.32	1.39
Inoculum (I)							
Probability		0.0038	0.1858	0.1572	0.9763	0.0855	0.6676
MSD[a]		0.03	11.40	7.07	3.70	0.42	0.44
MSD[b]		0.04	15.40	9.56	5.01	0.56	0.59
Interaction (D × I)		0.9667	0.8201	0.7745	0.4178	0.5197	0.4392

For each inoculum, means with different letters within a column are significantly different at $P < 0.01$.
Diet 1, F/C = 100/0 (all hay); diet 2, F/C = 90/10; diet 3, F/C = 80/20; diet 4, F/C = 70/30; diet 5, F/C = 60/40; diet 6, F/C = 50/50; tVFA, total volatile fatty acids (acetate + propionate + butyrate + isobutyrate + valerate + isovalerate); A/P, acetate/propionate ratio.
[a] Minimum significant difference at 5%.
[b] Minimum significant difference at 1%.

Table 4. Correlation (r^2) between rumen (RI) and faecal (FI) inocula for some *in vitro* fermentation data

Inoculum	OMCV/VFA (mL mmol l^{-1} g^{-1})	OMCV/dOM (mL mg^{-1})	VFA/dOM (mmol mg^{-1})
RI	0.8590**	0.8586**	0.5487NS
FI	0.7980*	0.7638*	0.8941**

Inoculum	dOM (mg)	OMCV (mL)	VFA (mmol)	R_{max} (mL h^{-1})
RI/FI	0.7244*	0.8674**	0.6720*	0.878*

OMCV, cumulative gas production related to incubated organic matter (OM) at 144 h; VFA, volatile fatty acids; dOM, OM degradability; R_{max}, maximum fermentation rate.
* $P < 0.05$; ** $P < 0.01$; NS, not significant.

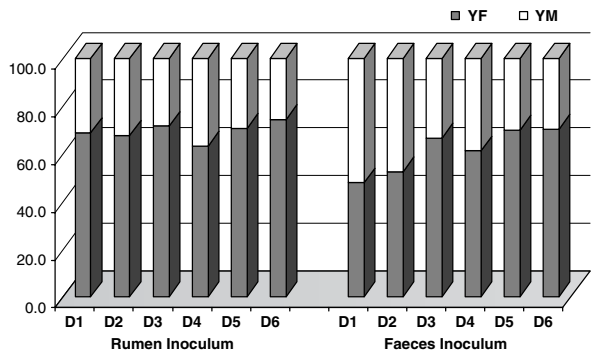

Figure 4. Fermented organic matter (OM) (Y_F) and OM utilised for microbial biomass yield (Y_M) on degraded OM basis (Y_D, %) in both inocula for diets differing in forage/concentrate (F/C) ratio: D1, F/C = 100/0 (all hay); D2, F/C = 90/10; D3, F/C = 80/20; D4, F/C = 70/30; D5, F/C = 60/40; D6, F/C = 50/50.

Several authors[9,51] found a negative correlation between gas production and substrate converted to microbial biomass. In our case, only with FI did gas production increase and microbial synthesis decrease from diet 1 to diet 6. However, the two parameters were not correlated for either inoculum. For FI the OM used for microbial growth showed very high values for diets 1 and 2 and an overall decreasing trend as the amount of concentrate in the diet increased. In FI the bacteria are both fewer and less active than in RI. Hence they may well use a greater amount of OM for their growth in conditions of energy deficiency (diets 1 and 2) compared with concentrate-rich diets (diets 3, 4, 5 and 6). This trend is evidenced by the lower rate of gas production[29] in the first 2 h of incubation for diets 1 and 2 (1.98 and 2.98 mL h^{-1} respectively) compared with diets 3, 4, 5 and 6 (4.14, 8.4, 6.87 and 6.80 mL h^{-1} respectively).

CONCLUSIONS

We found some significant differences in the *in vitro* fermentation characteristics of six ruminant diets with different forage/concentrate ratios between inocula of rumen fluid and faeces from sheep: lower OM degradability, higher gas and VFA production and faster fermentation kinetics with RI *vs* FI. Moreover, a

close relationship between the data (end-point and kinetics) was observed. The diet quality of the donor animal (only oat hay for 3 months) may well have affected the difference between RI and FI. In FI, increasing amounts of concentrate lead to decreasing microbial growth, estimated from VFA production using a stoichiometric equation. It was concluded that sheep faeces showed potential as an alternative to rumen liquor in studying fermentation using the *in vitro* technique. However, for the standardisation of faeces as inoculum for the gas production technique, further work is required: particular attention should be paid to the diet of donor animals, the time of incubation and the substrate to incubate (feed or diet).

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