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Rumen function and digestion parameters associated with differences between sheep in methane emissions when fed chaffed lucerne hay

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SUMMARY

An indoor experiment involving 10 rumen-cannulated Romney sheep was conducted in May and June 1998 at AgResearch Grasslands, Palmerston North, New Zealand, under restricted feeding conditions, in order to test the hypothesis that animal factors, in particular rumen fractional outflow rate (FOR) and rumen volume, have an influence on the between-sheep variation in methane (CH_4) emission. Sheep were fed 2-hourly on chaffed lucerne hay. Following an acclimatization period of 21 days, the experiment lasted 16 days. Energy and nitrogen (N) balances were measured on days 1–6. Cr-EDTA marker was continuously infused into the rumen from day 9 to 16, and rumen contents emptied and sampled on days 13 and 16. Particulate and fluid FOR were estimated using feed lignin and Cr-EDTA, respectively. Daily CH_4 production was measured by the sulphur hexafluoride tracer technique on days 2, 5, 6, 12 and 15 of the experiment.

CH_4 production (g/day) was positively correlated with the pool size of organic matter (OM) in the rumen (OM pool, g) ($r=0{\cdot}84$, $P=0{\cdot}002$), OM intake (OMI, g/day) ($r=0{\cdot}67$, $P=0{\cdot}04$), and the rumen fill (g, wet digesta) ($r=0{\cdot}76$, $P=0{\cdot}01$). Multiple regression analysis showed that CH_4 production was best predicted ($R^2=0{\cdot}88$) as a function of OM pool and the molar % of butyrate; however, OM pool alone accounted for a large proportion ($R^2=0{\cdot}71$) of the variation in CH_4 production.

CH_4 yield (% gross energy intake, % GEI) was negatively correlated with the particulate FOR (%/h) ($r=-0{\cdot}75$, $P=0{\cdot}01$) and buffering capacity of rumen fluid (mmol HCl) ($r=-0{\cdot}72$, $P=0{\cdot}02$), but positively correlated with the digestibility of cellulose ($r=0{\cdot}66$, $P=0{\cdot}04$). Multiple regression analysis showed that CH_4 yield was best predicted as a function of particulate FOR, OMI (g/kg liveweight$^{0{\cdot}75}$) and the molar % of butyrate ($R^2=0{\cdot}88$). Particulate FOR alone explained a large proportion ($R^2=0{\cdot}57$) of the variation in CH_4 yield. Particulate FOR was negatively correlated with rumen fill ($r=-0{\cdot}69$, $P=0{\cdot}03$) and digestibility of cellulose ($r=-0{\cdot}65$, $P=0{\cdot}04$).

These results suggest that sheep with lower rumen particulate FOR (i.e. longer rumen retention times) had larger rumen fills and higher fibre digestibilities and CH_4 yields. If rumen particulate FOR is to be used as a tool for CH_4 mitigation, the repeatability of its relationship to CH_4 emission must be assessed, preferably under grazing conditions.

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INTRODUCTION

The interaction between ruminant animals and rumen microorganisms is clearly symbiotic. The animal provides the microorganisms with a habitat for growth, whilst the microorganisms, in turn, provide the animal with the ability to digest plant cell wall carbohydrates and also provide nutrients such as volatile fatty acids and microbial protein (Hungate 1966). In the rumen fermentation reactions, reduction of carbon dioxide (CO_2) with hydrogen (H_2) and formation of methane (CH_4) by methanogens has a profound effect on the formation of different end-products, the amount of ATP generated and finally the efficiency of rumen microbial yield (van Nevel & Demeyer 1996). During fermentation, H_2 is formed in large amounts, but it does not accumulate because it is immediately used by methanogens (Wolin & Miller 1989). Thus, CH_4 formation may be seen as a major sink into which the H_2 from all rumen organisms drains (Demeyer & van Nevel 1975).

Although the end-products of rumen fermentation, such as CH_4, are the result of microbial activity and are influenced by the diet, it is recognized that animal factors such as mastication, salivation and digesta kinetics affect the rate and type of fermentation (Faichney 1993; Mathison *et al.* 1995; Wilson & Kennedy 1996; Varga & Kolver 1997). Thirty seven years ago, Blaxter & Clapperton (1965) showed that individual sheep fed on a common diet could differ significantly in their CH_4 yields and, more recently, Lassey *et al.* (1997) have reported that about 86% of the variation in daily CH_4 emission of grazing sheep is between-animal. Part of this variation might be genetic in origin, which might provide a tool for CH_4 emission control, but this possibility has not been studied.

Studies both with cattle (Hartnell & Satter 1979; Ørskov *et al.* 1988) and with sheep (Hodgson & Thomas 1975; Faichney 1993) have shown consistent between-animal differences in the rate of outflow of rumen digesta. In all these cases, animals with high outflow rates had smaller rumen volumes. Thus, the hypothesis tested in the current study was that between-animal differences in factors such as rumen fractional outflow rate and rumen volume have an influence on the between-sheep variation in CH_4 emission.

MATERIALS AND METHODS

Experimental design

An experiment involving 10 rumen-cannulated sheep was conducted in May and June 1998 at AgResearch Grasslands (Palmerston North, New Zealand), under controlled indoor conditions. There was an acclimatization period of 21 days to accustom the animals to the experimental conditions before a 16-day measurement period. Energy and nitrogen (N) balances were measured for a 6-day period (days 1–6), which was followed by a 2-day transition period (non-measurement) before an 8-day rumen digesta kinetics period (days 9–16). In this latter period, a chromium salt of ethylenediamine tetra-acetic acid (Cr-EDTA) was continuously infused into the rumen and rumen contents were bailed out on days 13 and 16. Daily methane production (g/day) was measured on days 2, 5, 6, 12 and 15 of the experiment.

Animals

The sheep were of the Romney breed, cryptorchids and aged 22 months. All sheep were fistulated in the rumen and fitted with permanent rubber cannulae (65 mm i.d.; Beruc Equipment Ltd., South Africa). Leakage of rumen contents was minimized by fitting plastisol washers around the cannulae.

The sheep were kept in digestibility crates and housed in a naturally well-ventilated building. Environmental conditions within the building were not measured, but outside mean ($\pm$S.D.) daily maximum and minimum temperatures were 15·0 ($\pm$2·29) and 6·8 ($\pm$3·59) °C, respectively, and the relative humidity was 87·6 ($\pm$8·26)%.

The sheep were weighed at the start and end of the 16-day measurement period and also immediately prior to emptying the rumen contents.

Feed and feeding

The sheep were fed on chaffed lucerne (*Medicago sativa*) hay. The total requirement of hay for the whole experiment was estimated prior to the experiment and after chaffing ($\sim$50 mm lengths) it was thoroughly mixed and the individuals' daily requirements were weighed and stored in plastic bags until required. Feeding was at a restricted level (1·2 times the maintenance energy requirements). Automatic overhead feeders delivered the day's ration in 12 feeds, at 2 h intervals. Drinking water was available *ad libitum*.

Fluid marker infusion procedure

The fluid-phase marker Cr-EDTA was prepared by the method of Binnerts *et al.* (1968) and adjusted to pH 6·7. The infusate, containing 380 μg Cr/ml was continuously infused via the rumen cannulae for 8 days at a nominal rate of 0·53 ml/min using a peristaltic pump (PLG-multipurpose pump; Dasaga, Heidelberg, Germany). The actual infusion rate was determined for each sheep.

Sample collection procedures

Energy and N balances

Samples of feed on offer were taken prior to the experiment from the feed batch, before the individual

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daily rations were weighed. After pooling and mixing the samples obtained, two subsamples were taken for dry matter (DM) determination (100 °C, 48 h). Two other subsamples were stored at −20 °C for chemical analysis.

The design of the digestibility crates allowed the automatic separation of faeces and urine. Faeces were collected onto a meshed tray and a steel chute mounted below the tray served to direct urine into buckets containing sufficient H_2SO_4 (1·8 M) to decrease the pH to between 2·5 and 3·0.

The amounts of daily feed refusals and faeces outputs were recorded and subsamples (10%) taken for DM determinations (100 °C, 48 h). Other daily subsamples (10%) of feed refusals and faeces were stored frozen (−20 °C). After the collection, all frozen subsamples were pooled within animals, mixed thoroughly, re-sampled, then freeze-dried, ground through a 1 mm mesh sieve (Wiley Mill, USA) and used for analysis.

Daily urine production was recorded and samples (10%) were diluted (1:3, v/v) with water, subsampled (10%) and stored (−20 °C) for later analysis of purine derivatives (PD) on samples pooled within sheep. Other samples of the daily urine production (10%) were taken, stored frozen (−20 °C) and later pooled within sheep, freeze-dried and analysed for energy and N contents.

Sampling of rumen contents

Sheep reticulo-rumens (hereafter named rumen) were emptied (bailed) on days 13 (morning) and 16 (afternoon). Rumen bailings took place within 30 and 60 min after the feed delivery (09.00 h on day 13 or 15.00 h on day 16). Rumen contents were weighed, thoroughly mixed, and sampled before being returned to the rumen. The procedure took about 7 min per animal. Subsamples of digesta were taken for triplicate DM determination (60 °C, 72 h). Other subsamples of digesta were taken and managed in the following way: (1) ∼200 g was used for immediate pH determination, then stored (−20 °C), freeze-dried, ground to pass through a 1 mm sieve and used for chemical analysis, (2) ∼100 g was stored (−20 °C) and later used for particle size determinations, (3) ∼100 g was stored (−20 °C) for later analysis of Cr concentration, (4) ∼100 g was strained through a nylon bag (60 μm mesh) and samples taken for analysis of ammonia (NH_3) and volatile fatty acid (VFA) concentrations, protozoa counting and measurement of buffering capacity.

The rumen fluid samples for NH_3 and VFA analysis were acidified, deproteinized and centrifuged immediately after sampling, using procedures described by Domingue *et al.* (1991). Samples for protozoa counting were prepared according to Odenyo *et al.* (1997): 4 ml of strained rumen fluid was added to 16 ml of formal-saline solution (8·1 g NaCl and 100 ml formaldehyde (37% w/v) per litre) and kept at 4 °C until counting.

CH_4 production measurement

The sulphur hexafluoride (SF_6) tracer technique (Johnson *et al.* 1994) was used for daily CH_4 production (g/day) measurements. This technique involves dosing each animal with a permeation tube containing SF_6, which is calibrated to release 1–2 mg of SF_6 over 24 h. Exhaled gas is collected continuously from near the nose via a plastic tube attached to a halter and leading to an evacuated PVC yoke. Thus an integrated 24 h breath-sample is collected from each participating animal and subsequently analysed by gas chromatography for both CH_4 and SF_6. All the measurements were carried out while sheep were kept in digestibility crates. Crates were placed 2–3 m from each other within the building. The PVC gas collection yokes were suspended towards the rear of the digestibility crates and a lengthened sample line from the halter to the yoke was closely attached to the animal's back line to prevent chewing.

Laboratory methods

Samples of feed offered, feed refusals, faeces and urine were analysed for gross energy (GE) content using an adiabatic bomb calorimeter (Gallenkamp Autobomb – Automatic; London, UK) and for total N by the Kjeldahl method. Organic matter (OM) content of feed on offer, refusals, faeces and rumen contents was determined by ashing in a furnace at 550 °C for 16 h. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents of feed, feed refusals, faeces and rumen contents were determined according to the methods of Goering & van Soest (1970). Hemicellulose was calculated as NDF–ADF, whereas cellulose was calculated as ADF–ADL. Chromium concentration in the rumen digesta was analysed using an Inductively Coupled Plasma Emission Spectrometer (ARL 34000) after digestion with concentrate nitric acid.

Urinary purine derivatives (PD), allantoin, xanthine (plus hypoxanthine) and uric acid were respectively determined using the colorimetric, enzymatic and uricase methods of Chen & Gomes (1992).

The pH of rumen contents was determined using a 3020 pH Meter (Jenway Ltd., England). VFA concentrations in rumen fluid were determined by gas chromatography (HRGC 5380, Carlo Erba Instruments, Italy) as described by Hoskin *et al.* (1995). NH_3 concentration in rumen fluid was determined by auto analyser (COBAS, FARA, Basel, Switzerland), using commercially available diagnostic kits (Sigma, St. Louis, USA), which are based on the principle of reductive amination by L-glutamate dehydrogenase.

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Buffering capacity of rumen fluid was determined according to the method described by Ding *et al.* (1997). Hydrochloric acid (HCl, 1 M) was added, in quantities of 20 µl at a time, to a 20 ml sample of rumen fluid until pH 5·5 was reached. In this pH range (~7·0–5·5), buffering capacity was defined as the amount of acid (mmol/l) required to change the pH of 1 ml of rumen fluid by 1 unit (Ding *et al.* 1997).

The particle size distributions in rumen digesta were determined using a wet sieving apparatus (Turner & Newall Ltd., England) and following the procedure described by Domingue *et al.* (1991). The sieve sizes (length of side of square hole) used were 4·0, 2·0, 1·0, 0·5 and 0·25 mm. Materials retained on the sieves were washed onto weighed filter paper (Whatman No. 21) in a Buchner funnel and dried at 60 °C to constant weight to determine the dry weight of each particle size fraction. The dry weight of material not retained on the sieves (<0·25 mm particles) was determined by difference from the initial sample dry weight and the sum of recovered particulate DM fractions. Results for each fraction (particulate and soluble) were expressed as the % of the total initial DM in each sample.

For protozoa counting, a 1-ml aliquot of the formalin-treated rumen fluid sample was pipetted with a wide-orifice pipette into a 20 ml beaker containing 9 ml tap water (1:10 dilution). The diluted sample was pipetted into a counting chamber with a wide-orifice pipette. Protozoa were counted at a magnification of 128×. Each sample was counted in triplicate, and each counting involved 15 fields. The total number of protozoa was counted, and the numbers of holotrichs and entodiniomorphs were also recorded. Protozoa counts were expressed per ml of rumen fluid.

Calculations

Daily feed intake and CH_4 emission

The mean daily intakes of DM (DMI), OM (OMI) and GE (GEI) measured during the 6-day balance period were assumed to be the same during the rumen digesta kinetics period.

In the study, the absolute daily production of CH_4 (g/day) is named 'CH_4 production', whereas the proportion of the daily GEI (% GEI) lost as CH_4 energy is named 'CH_4 yield'. A broad term, 'CH_4 emission', is used here to refer to CH_4 production, CH_4 yield and CH_4 production rate per unit of intake.

Rumen fill and apparent mean retention time (AMRT) in the rumen

Rumen fill was measured as the weight (g) of the wet digesta per animal upon bailing.

In order to express the DM fractions (g) of the rumen digesta as a proportion of their respective intakes (g/h), the apparent mean rumen retention time (AMRT, h) (Minson 1966) of these constituents was calculated.

Fractional outflow rate (FOR) and mean retention time (MRT) in the rumen

Rumen FOR (%/h), the proportion of a digesta constituent that leaves the rumen per unit time (Faichney 1980), was calculated using the continuous infusion and total sampling procedure of Faichney (1975). Liquid FOR was calculated with reference to the external marker Cr-EDTA, whereas the internal marker ADL was used to calculate particulate FOR (Domingue *et al.* 1991). No correction for Cr-EDTA absorption from the rumen was made on the assumption that it was less than <1·7% of daily dose (Goodall & Kay 1973) and it was assumed that the faecal output of ADL represented its abomasal flow (Faichney 1980).

The MRT (h) of the liquid and the particulate phases in the rumen were calculated as the reciprocal of their respective FOR (Faichney 1980).

Digesta particle size distribution and modulus of fineness

The particle size of rumen digesta was expressed both as 'modulus of fineness' and as 'cumulative DM'. Modulus of fineness was calculated according to the procedure described by Poppi *et al.* (1980). Alternatively, the cumulative proportions (% total DM) and pool size of particles >1 mm and <1 mm in the rumen were calculated. According to Poppi *et al.* (1980) the critical particle size for clearance from the rumen is about 1·2 mm.

Microbial N supply from the rumen

Based on urinary PD excretion, the microbial N supply (g N/day and g N/kg digestible organic matter apparently fermented in the rumen (DOMR)) was calculated according to the procedures described by Chen & Gomes (1992). Briefly, the total PD excretion (sum of allantoin, uric acid, xanthine and hypoxanthine; mmol/day) was calculated. Then, based on the daily excretion of PD (and accounting for the endogenous contribution of PD), the amount of microbial purines absorbed (mmol/day) was estimated assuming that 84% of absorbed purines were recovered as PD in urine. Microbial N supply (g N/day) was calculated assuming: (1) the N content of absorbed purines was 70 mg/mmol, (2) the digestibility of microbial purines was 83% and (3) the ratio of purine-N:total N in mixed rumen microbes was 11·6:100.

Statistical analysis

Data for feed intake, energy and N balances, CH_4 emission and for variables derived from the two

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rumen bailings were pooled for each sheep and the mean values and standard deviations (S.D.) calculated.

The between-sheep variation in CH_4 emission was calculated by fitting sheep as class in the GLM procedure of SAS (SAS 1985). For this, all the daily CH_4 emission values (5 per sheep) were used.

The relationships between the CH_4 production or CH_4 yield and each measured variable, were assessed by correlation analysis (SAS 1985). In addition, multiple regression analysis of CH_4 production or CH_4 yield, upon the other variables was carried out using the forward model-selection method of the stepwise procedure of SAS (1985). In order to guard against the selection of too many variables in the multiple regression model, the level of significance for the SLENTRY criteria for the stepwise procedure (SAS 1985) was set at $P<0{\cdot}10$. The aim of the multiple regression analysis was to identify the most important variables responsible for between-animal variation in CH_4 production or yield. Thus, intake, rumen pool size, AMRT and apparent digestibility of the digesta constituent (e.g. OM, DM) with the highest partial correlation with CH_4 production or CH_4 yield were included.

In the multiple regression analysis, the variables included in the model-selection procedure were almost the same for CH_4 production and CH_4 yield. Exceptions were that in the multiple regression analysis of CH_4 yield, the variables feed intake per kg liveweight$^{0{\cdot}75}$ ($LW^{0{\cdot}75}$), microbial N supply (g/kg DOMR) and AMRT (h) were also included. When not otherwise stated, the number of observations was 10 (10 sheep).

RESULTS

Feed intake, energy and N balances and rumen digestion characteristics

The sheep maintained their LW throughout the experiment (mean (±S.D.) LWs at the start and end of experiment were 46·8 (±5·35) and 46·9 (±4·81) kg, respectively). The feed contained (per kg DM), 30·2 g N, 423 g NDF, 359 g ADF, 73 g lignin, 94 g ash and 18·35 MJ of GE and mean (±S.D.) daily intakes of DM (g), OM (g) and GE (MJ) were 1083 (±114·5), 987 (±92·3) and 19·9 (±2·10), respectively. The mean (±S.D.) apparent digestibilities of DM, OM and GE were 0·591 (±0·0182), 0·604 (±0·0192) and 0·567 (±0·0190), respectively. The between-sheep coefficient of variation (CV = S.D./mean) for feed intake was 10%. The corresponding CV for the apparent digestibilities was relatively small (3%).

Metabolizable and faecal energy represented 45·9 (8·4 MJ ME/kg DM) and 43·4%, respectively of the GE intake (Table 1). Energy losses in urine and CH_4 were similar to each other. Urinary excretion represented a loss of 67·4% of N intake (Table 1). A large variation was observed in the retention of N (range: −3·8 to 13·6% of N intake) and also in microbial N supply from the rumen (g/day or g/kg DOMR) (Table 1).

Except for protozoa counts, the variations (CV) in rumen pH, NH_3 concentration, concentrations and molar proportions of VFA and buffering capacity of rumen fluid were relatively small (Table 2). Almost 95% of the protozoal population were entodiniomorphs, these being mostly small *Entodinium*.

Table 1. *Mean (±S.D.) daily balances and partitioning (% of intake) of energy (MJ) and nitrogen (N, g), and microbial N supply from the rumen (g/day and g/kg DOMR)*

	Balance and partitioning	
Energy	MJ/day	% of intake
Intake	19·9±2·10	100
Excretion		
Faeces	8·6±1·11	43·3±1·91
Urine	1·1±0·08	5·6±0·33
CH_4	1·0±0·08	5·2±0·51
Metabolizable	9·1±1·30	45·9±1·89
N	g/day	
Intake	32·9±2·88	100
Excretion		
Faeces	9·4±1·10	28·5±1·38
Urine	22·2±2·68	67·4±3·55
Retained	1·3±1·29	4·1±4·20
Microbial N supply		
g/day	11·5±2·40	
g/kg DOMR*	29·6±4·92	

* DOMR, digestible organic matter apparently fermented in the rumen, estimated as 0·65 DOMI, digestible OM intake (Chen & Gomes 1992).

Table 2. *Mean (±S.D.) values for rumen fermentation parameters (pH, NH_3, VFA), buffering capacity of rumen fluid and protozoa counts*

	Mean±S.D.
pH	6·8±0·06
NH_3 (mg/l)	284±18·2
VFA (mmol/l)	119±13·4
Acetate (mol %)	68·4±0·73
Propionate (mol %)	19·0±0·90
Butyrate (mol %)	8·2±0·30
Acetate/propionate	3·6±0·24
Buffering capacity (mmol HCl)	39·3±2·11
Protozoa counts (10^5/ml)	
Holotrichs	0·25±0·082
Entodinomorphs	4·61±0·951
Total	4·86±0·981

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Table 3. *Mean (±S.D.) rumen fill, pool sizes and particle size distribution of rumen digesta*

	Mean ± S.D.
Rumen fill (g, wet digesta)	4790 ± 609·8
Rumen pool size (g)	
Liquid	4243 ± 518·8
Dry matter (DM)	546 ± 98·6
Organic matter (OM)	482 ± 90·2
Neutral detergent fibre (NDF)	303 ± 64·7
Acid detergent fibre (ADF)	238 ± 50·1
Hemicellulose	65 ± 15·9
Cellulose	163 ± 34·7
Lignin	75 ± 15·0
Particle size distribution in rumen	
Particles > 1·00 mm	
pool size (g DM)	129 ± 43·0
% (of total DM pool)	23·2 ± 5·23
Particles < 1·00 mm	
pool size (g DM)	417 ± 69·2
% (of total DM pool)	76·8 ± 5·21
Modulus of fineness	2·4 ± 0·17

Table 4. *Mean (±S.D.) values for liquid and particulate fractional outflow rates (FOR), liquid and particulate mean retention times (MRT) and apparent mean retention times (AMRT) of some digesta constituents**

	FOR (%/h)	MRT (h)	AMRT (h)
Liquid	13·6 ± 2·78	7·7 ± 1·47	
Particulate	4·1 ± 0·80	25·4 ± 4·75	
DM			12·4 ± 1·83
OM			12·0 ± 1·78
NDF			16·6 ± 3·53
ADF			15·3 ± 3·11
Hemicellulose			25·3 ± 8·22
Cellulose			13·0 ± 2·60
Lignin			24·5 ± 6·17

* For abbreviations refer to Table 3.

The mean rumen fill (Table 3) represented 10·4% of the LW of the animal. The liquid component of the rumen fill accounted for 88·5% of the total contents. Particles below 1·0 mm size accounted for 76·8% of the rumen DM content and the modulus of fineness of the particles (>0·25 mm) was 2·4 (Table 3).

The mean liquid FOR from the rumen was 3·3 higher than the mean particulate FOR (Table 4) and the variations (CV) for these variables were 20%. The AMRT values of digesta constituents (Table 4) indicate that hemicellulose and lignin were the most recalcitrant components for rumen clearance.

The mean (±S.E.) daily production of CH_4 was 18·7 (±0·72) g per sheep and it accounted for 5·2 (±0·14)% of CH_4 yield (% GEI). Variation between sheep represented 70 and 62% of the total variation in CH_4 production and CH_4 yield, respectively.

Relationships between CH_4 emission and the other rumen digestion variables

Table 5 shows the coefficients of correlation (r) between CH_4 production (g/day) or CH_4 yield (% GEI) and the other measured variables. CH_4 production was positively related to OMI (g/day; $P = 0{\cdot}04$), OM pool size in the rumen (OM pool, g; $P = 0{\cdot}002$) and rumen fill (g; $P = 0{\cdot}01$). The correlations between CH_4 production and urinary N (% of N intake), microbial N supply (g/day), and molar proportion of butyrate (Butyrate, mol %) were positive, but only approached statistical significance ($P \approx 0{\cdot}10$).

CH_4 yield (% GEI) was negatively related to the particulate FOR (%/h; $P = 0{\cdot}01$) and buffering capacity of rumen fluid (mmol HCl; $P = 0{\cdot}02$), but positively related to the AMRT of organic matter (AMRT of OM, h; $P = 0{\cdot}03$) and the digestibility of cellulose ($P = 0{\cdot}04$). No significant relationships were found between CH_4 yield and the apparent digestibility of other dietary constituents.

The results from the multiple regression analysis were as follows.

(*a*) *CH_4 production*

From all the variables included, only two variables were selected into the regression model to best explain the variation in CH_4 production. The first variable selected was OM pool (g), which explained 71% of the total variation (Fig. 1). The second variable selected to enter the model was Butyrate (mol %), which increased the variation explained by the model to 88%. Thus, the multiple regression model to best explain CH_4 production is shown by the equation (±S.E.)

$$\begin{aligned} &CH_4 \text{ production (g/day)} \\ &\quad = -14{\cdot}4 + 0{\cdot}02\ (\pm 0{\cdot}003)\ \text{OM pool (g)} \\ &\qquad + 2{\cdot}91\ (\pm 0{\cdot}898)\ \text{Butyrate (mol\%)}; \\ &R^2 = 0{\cdot}88;\ P = 0{\cdot}0005 \end{aligned} \quad (1)$$

(*b*) *CH_4 yield*

Of all the variables included, three were selected into the regression model to best explain the variation in CH_4 yield. The first variable to be selected was the particulate FOR (%/h) which explained 57% of the total variation (Fig. 2). The second variable selected was OMI (g/kg $LW^{0{\cdot}75}$), which together with particulate FOR explained 73% of the variation in CH_4 yield. The third and last variable selected to enter the model was Butyrate (mol%), which together with the

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Table 5. *Coefficients of correlation* between the CH_4 production (g/day) or CH_4 yield (% GEI) and the other measured variables†*

	CH_4 production (g/day)	CH_4 yield (% GEI)
OMI (g/day)	0·67	
Rumen OM pool (g)	0·84	
Rumen fill (g, wet digesta)	0·76	
Rumen particulate FOR (%/h)		−0·75
Buffering capacity (mmol HCl)		−0·72
AMRT of OM (h)		0·70
Digestibility of cellulose		0·66

* For variables: feed intake, rumen pool size, AMRT and apparent digestibility, only the digesta constituent with the highest correlation coefficient was tabulated. Autocorrelated variables (e.g. FOR *v.* MRT) and non-significant ($P>0·05$) correlations were not tabulated.
† For abbreviations refer to previous tables.

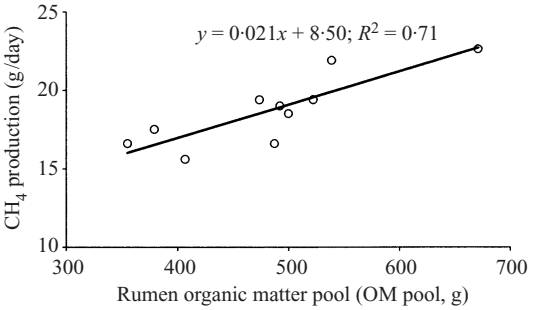


Fig. 1. Relationship between the rumen organic matter pool size (OM pool, g) and daily CH_4 production (g/day).

other two variables accounted for 88% of the variation in CH_4 yield. The multiple regression model to best explain the variation in CH_4 yield is as shown by the equation (±S.E.)

$$\begin{aligned} CH_4 \text{ yield (\% GEI)} &= 4·21 - 0·36\ (\pm 0·08)\ \text{Particulate FOR (\%/h)} \\ &\quad - 0·03\ (\pm 0·01)\ \text{OMI (g/kg LW}^{0·75}\text{)} \\ &\quad + 0·52\ (\pm 0·20)\ \text{Butyrate (mol\%)}; \\ R^2 = 0·88;\ P = 0·004 \end{aligned} \quad (2)$$

DISCUSSION

The variation (CV) in the daily feed intake was similar to the variation in LW. Even though sheep were fed at 1·2 times their maintenance requirements, the between-sheep variation (CV) in daily feed intake

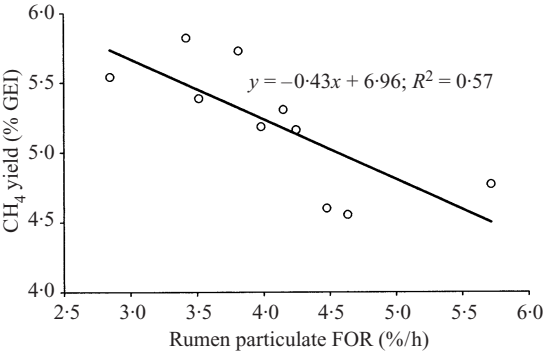


Fig. 2. Relationship between the rumen particulate fractional outflow rate (particulate FOR, %/h) and daily CH_4 yield (% GEI).

per kg $LW^{0·75}$ was still 7%, a consequence of the differences between-sheep in the ratio of feed DM eaten to that offered, which ranged from 0·88 to 0·98. Nevertheless, a high correlation ($r=0·71$, $P=0·02$) was found between the absolute daily feed intake and $LW^{0·75}$.

In general, the rumen digestion parameters were in the range reported in the literature for sheep fed on lucerne hay (Egan *et al.* 1975; Ulyatt *et al.* 1984; Domingue *et al.* 1991; Nandra *et al.* 1993; de Vega *et al.* 1998). The NH_3 concentration in the rumen fluid was well above the value of 190 mg NH_3-N, the suggested threshold required for optimal fibre digestion (Mehrez *et al.* 1977). Nevertheless, two sheep approached a negative N balance (<1% of N intake) as a consequence of low feed intake.

The between-sheep variation in daily CH_4 production observed in this study is slightly lower than that (86%) reported by Lassey *et al.* (1997) for 50 grazing sheep, but within the range cited by Ulyatt *et al.* (1999). The restricted and controlled feeding conditions imposed in the present study probably contributed to the lower between-sheep variation in CH_4 production. The between-sheep variation in daily CH_4 yield observed in this study was ±0·447% GEI and represented 8·6% of the mean, a variation (CV) similar to those (7·2 and 8·1%) reported by Blaxter & Clapperton (1965) for CH_4 measurements carried out in respiration calorimeter chambers.

Methane production was positively and significantly ($P<0·05$) related to OMI (g/day), to rumen OM pool (g), and to rumen fill (g) (Table 5). In agreement with the observations of Purser & Moir (1966), rumen OM pool was positively related not only to the OMI ($r=0·65$, $P=0·04$), but also to the rumen fill ($r=0·94$, $P=0·0002$). These relationships suggest that larger feed intakes were associated with an increased physiological capacity of the rumen, which allowed longer retention times and higher digestion rates

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(Grovum 1984), thus resulting in larger CH_4 production.

It is well established (Blaxter & Clapperton 1965) that CH_4 production (g/day) increases with absolute feed intake and this was confirmed in the present study (Table 5). Nevertheless, in the multiple regression model of CH_4 production (Eqn 1), OM pool (g) was identified as being more important than the absolute feed intake (OMI, g/day) and rumen fill. This was possibly due to the restricted feeding level imposed in the study.

Methane production was positively, but not significantly, correlated with urinary N (% of N intake; $r=0{\cdot}60$, $P=0{\cdot}07$). It has been shown that the rumen protozoal population contributes positively to CH_4 production (Jouany & Lassalas 2000) and urinary N loss (Jouany 1995). Nevertheless, no relationship between CH_4 production and protozoa count was found in the present study, and urinary N loss was not selected in the multiple regression model of CH_4 production.

Methane production was not related to either the concentrations or molar proportions of acetate or propionate, but it was positively and weakly correlated ($r=0{\cdot}54$, $P=0{\cdot}10$) with the molar proportion of butyrate, a relationship which was also reported by Whitelaw *et al.* (1984). Acetate and butyrate formation in the rumen provide the major sources of H_2 for methanogenesis (Wolin 1960) and acetate is considered to be the major precursor of butyrate during rumen fermentation (Russell & Wallace 1989). In the multiple regression analysis of CH_4 production, butyrate (mol %) was the second variable selected in the model (Eqn 1) and accounted for 7·4% of the variation in CH_4 production. Rumen ciliates have been associated with an increased concentration of butyrate in the rumen fluid (Whitelaw *et al.* 1984; Hegarty *et al.* 1994; Jouany & Lassalas 2000), but no evidence for this was found in the present study. However, it is possible that the straining of rumen digesta through a nylon bag (60 μm mesh) might have excluded some protozoa from the samples of rumen fluid. Butyrate (mol %) was also selected (ranked third and last) in the multiple regression model of CH_4 yield (Eqn 2), explaining an extra 14·2% of the total variation.

Methane yield was positively and significantly ($P<0{\cdot}05$) related to AMRT of OM (h) and to the apparent digestibility of cellulose (%), but negatively and significantly ($P=0{\cdot}01$) related to the particulate FOR (%/h) (Table 5). These relationships suggest that longer retention times of feed in the rumen were associated with greater digestibility of cell walls and therefore greater CH_4 yields. However, from the latter variables, the particulate FOR was identified by the multiple regression analysis as having the strongest relationship to CH_4 yield (Eqn 2), explaining 57% of the total variation (see Fig. 2). The influence of particulate FOR on CH_4 yield is in agreement with previous observations (Demeyer & van Nevel 1975; Okine *et al.* 1989), i.e. the higher the particulate FOR, the lower the CH_4 yield due to the shorter time that feed particles are exposed to microbial fermentation.

Methane yield was negatively correlated ($P=0{\cdot}02$) with the buffering capacity of rumen fluid (Table 5). In this study, lucerne hay was the sole feed and no between-sheep variation in pH was observed (CV < 1%). Therefore, under these conditions it is unlikely that buffering capacity directly influenced CH_4 yield, but probably reflected the relationship between the rumen particulate FOR and CH_4 yield. Saliva is an important source of buffer in the rumen system (Ding *et al.* 1997) and the rate of saliva production influences the rumen dilution rate (Harrison *et al.* 1975; Sibanda *et al.* 1997). In the present study, buffering capacity was not correlated to liquid FOR ($r=0{\cdot}26$, $P=0{\cdot}46$), but was positively and weakly correlated ($r=0{\cdot}57$, $P=0{\cdot}08$) to particulate FOR. Buffering capacity was not identified in the multiple regression model of CH_4 yield as accounting for more variation than particulate FOR alone.

From the apparent digestibilities of dietary constituents, only the digestibility of cellulose was correlated ($P=0{\cdot}04$) with CH_4 yield, in agreement with the knowledge that cellulose is the most methanogenic carbohydrate (Moe & Tyrrell 1979). The positive relationship between the digestibility of cellulose and CH_4 yield is in agreement with the observations of Blaxter & Clapperton (1965) for a restricted feeding level (an effect of longer rumen retention time). However, the digestibility of cellulose was not selected in the multiple regression model of CH_4 yield, probably because its effect was overshadowed by that of particulate FOR.

The correlation between the CH_4 yield and OMI per kg of $LW^{0{\cdot}75}$ approached significance ($r=-0{\cdot}52$, $P=0{\cdot}12$). OMI (g/kg $LW^{0{\cdot}75}$) was the second variable selected to enter the multiple regression model of CH_4 yield (Eqn 2), accounting for an extra 16% of the total variation. CH_4 yield decreased as OMI (g/kg $LW^{0{\cdot}75}$) increased, a relationship also observed in other studies (Blaxter & Clapperton 1965; Pelchen & Peters 1998).

The quantitative interaction between the fractional rates of digestion and passage determines the digestibility in the rumen (Poppi *et al.* 2000). In the present study, with restricted feeding, particulate FOR (%/h) was the major factor involved in the between-sheep variation in CH_4 yield. Particulate FOR not only correlated negatively with rumen fill (weight of wet digesta) ($r=-0{\cdot}69$, $P=0{\cdot}03$), but also with cellulose digestibility ($r=-0{\cdot}65$, $P=0{\cdot}04$). Thus, larger rumen fills were associated with longer retention times of feed in the rumen and consequently greater fibre digestibilities and CH_4 yields. Whether these interrelationships, observed at restricted feeding conditions, are the same under *ad libitum* feeding conditions

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(e.g. generous pasture allowance at grazing), is unknown.

In conclusion, although methane is produced by microbes, the study has demonstrated that rumen particulate fractional outflow rate (particulate FOR), an animal factor, explained a large part of the between-sheep variation in CH_4 yield. If this relationship was persistent under *ad libitum* feeding in the long-term and the rumen particulate FOR was heritable, genetic selection might be effective as a tool to reduce livestock CH_4 emission.

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