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Archaeal abundance in *post-mortem* ruminal digesta may help predict methane emissions from beef cattle

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Methane produced from 35 Aberdeen-Angus and 33 Limousin cross steers was measured in respiration chambers. Each group was split to receive either a medium- or high-concentrate diet. Ruminal digesta samples were subsequently removed to investigate correlations between methane emissions and the rumen microbial community, as measured by qPCR of 16S or 18S rRNA genes. Diet had the greatest influence on methane emissions. The high-concentrate diet resulted in lower methane emissions ($P < 0.001$) than the medium-concentrate diet. Methane was correlated, irrespective of breed, with the abundance of archaea (R = 0.39), bacteria (−0.47), protozoa (0.45), Bacteroidetes (−0.37) and *Clostridium* Cluster XIVa (−0.35). The archaea∶bacteria ratio provided a stronger correlation (0.49). A similar correlation was found with digesta samples taken 2–3 weeks later at slaughter. This finding could help enable greenhouse gas emissions of large animal cohorts to be predicted from samples taken conveniently in the abattoir.

M ethane is a greenhouse gas with a global warming potential 25-fold that of carbon dioxide[1]. Ruminant livestock production, through the fermentation of feed in the gut, contributes significantly to greenhouse gas production by agriculture. In the UK in 2005, CH_4 accounted for 37% of all agricultural emissions[2]. Methane production also represents a loss of energy (from 2 to 12% of gross energy intake[3]), which could otherwise be available for growth or milk production. Preventing methane emissions therefore would benefit the environment and the efficiency of livestock production. A large majority of the methane produced by cattle is derived from the rumen[4], which harbors a population of methanogenic archaea that convert the H_2 and CO_2 produced by a complex community of ciliate protozoa, bacteria and anaerobic fungi to methane[4,5]. A massive worldwide research effort has investigated various mitigation strategies, particularly feed additives that might inhibit H_2 production, provide an alternative H sink or inhibit the growth of archaea[2,5]. Other strategies include chemogenomics, immunization and genetic selection[2,5–7]. Determining the effectiveness of these interventions on methane emissions is generally expensive and labor-intensive. The 'gold-standard' method of enclosing individual animals in respiration chambers and measuring methane concentrations in gas leaving the chamber does not lend itself to the screening of more than a few animals. Here we demonstrate a proxy method whereby methane emissions from individual animals can be related to the concentration of archaea in ruminal digesta, not only taken from the live animal but also taken 2–3 weeks later at slaughter.

Results

Diets, breeds and methane emissions. Thirty-six Aberdeen Angus cross and 36 Limousin cross steers received two diets, one mainly concentrate-based (high-concentrate) and the other a forage-concentrate-based diet (medium-concentrate) (Table 1). Four animals were removed from the experiment for different reasons. The volumes of methane produced daily by the Aberdeen Angus cross steers were higher ($P < 0.05$) than the Limousin cross steers (Table 2). Aberdeen Angus steers consumed more feed, however, and when methane volumes were compensated for feed intake, there were no significant differences between breeds. Diet played a major role in methane emissions, with the medium-concentrate diet producing higher methane emissions when expressed per unit of dry matter or energy intake (Table 2). Within each breed-diet group, however, there were large differences between individual animals (Fig. 1).

Table 1 | Ingredient composition (fresh weight basis; kg/tonne) and chemical analysis of medium- and high-concentrate diets offered to cattle

Ingredient	Medium-concentrate	High-concentrate
Barley straw		81
Grass silage	413	
Whole crop barley silage	340	
Barley	156	688
Maize distiller's dark grains	86	200
Molasses		20
Minerals and vitamins supplement	5	10

	Barley	MDDG	Silage	WCBS	Straw
Dry matter (g/kg)	850	865	211	329	825
(g/kg DM)					
Ash	22	47	67	60	37
Crude protein	104	273	147	111	21
ADF	69	216	345	312	519
NDF	163	377	567	540	826
Starch	592	22	6	141	3
pH			3.9	4.7	
Gross energy (MJ/kg DM)	18.8	21.8	19.0	19.1	17.1

Minerals (Beef Special, Norvite, Insch, Aberdeenshire, UK) contained (mg/kg), Fe, 6036; Mn, 2200; Zn, 2600; Iodine, 200; Co, 90; Cu, 2500; Se 30; (µg/kg): vitamin E, 2000; vitamin B12, 1000; vitamin A, 151515; vitamin D, 2500.
Molasses had a DM of 688 g/kg and gross energy of 15.3 MJ/kg DM.
ADF, NDF; acid and neutral detergent fiber.
MDDG, maize distillers dark grains; WCBS, whole crop barley silage.

Figure 1 | Variation in methane production by cattle according to breed and diet. Each category comprises data from a single breed/diet combination: AA(C), Aberdeen Angus cross steers on the high-concentrate diet; Lim(C), Limousin cross steers on the high-concentrate diet; AA(M), Aberdeen Angus steers on the medium-concentrate diet; Lim(M), Limousin steers on the medium-concentrate diet. The horizontal line within each box represents the mean of all the daily methane emission measurements in units of g methane per kg DM intake. The two segments in the box represent the second and third quartiles, while the lines describe the first and fourth quartiles. Thus, the maximum for AA(C) was 18.1 and the minimum 7.6, with a mean of 13.6 g methane/kg DM intake.

Specificity and coverage of archaeal qPCR primers. Extracted DNA was analysed by qPCR of 16S rRNA genes. Archaeal DNA was amplified using the universal archaeal primers Met630F and Met803R[8]. The archaeal primers were tested *in silico* for coverage following bias revealed in some primer sets[9] and the latest revelations about the RCC clade[10], related to Thermoplasmatales and provisionally named Methanoplasmatales[11]. The primers were found to be genuinely specific for archaea and comprehensive for the major orders of archaea found in the rumen (Fig. 2).

Microbial abundances in ruminal digesta. Total bacterial copy numbers of 16S rRNA genes per ng extracted DNA were 30% lower in digesta taken from the medium-concentrate diet, and were also 40–47% lower in samples taken *post mortem* (Table 3). Protozoa were more than twice as abundant in the medium-concentrate diet (Table 3). The largest effect of diet on the abundance of the subsets of the bacterial community was seen in a >50% increase of the proportion of *Clostridium* cluster IV, expressed as a proportion of total bacteria, in the medium-concentrate diet (Table 3). Archaea were similarly >50% more abundant in the
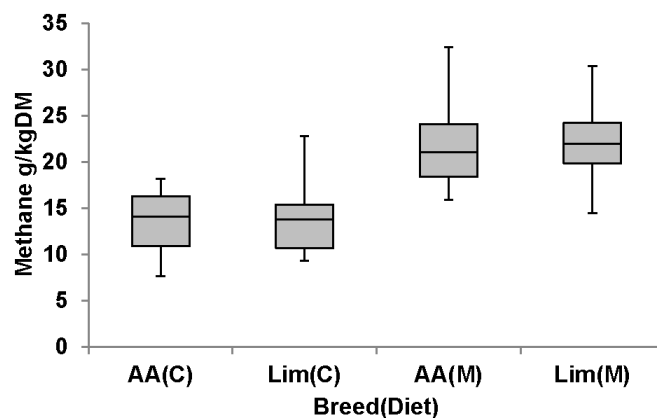
medium-concentrate diet (Table 3). The abundance of Bacteroidetes in the high-concentrate diet was similar to the sum of the two *Clostridium* clusters, but was lower in the medium-concentrate diet (Table 3).

A correlation matrix (Table 4) was drawn up to compare how the proportions of the different microbial groups varied relative to each other. The abundance of protozoa across all samples from live animals was negatively correlated with the abundance of total bacteria and of *Clostridium* cluster XIVa and Bacteroidetes. Archaea were also negatively correlated with total bacteria and Bacteroidetes in particular. However, they were positively correlated with *Clostridium* cluster IV (Table 4).

Microbial abundances and methane emissions. Based on the results from qPCR of extracted DNA, the abundance of archaea in extracted DNA tended to be linked positively to methane emissions, but the relationship was weak (Fig. 3, Table 4). An inverse correlation, also weak, was found between methane emissions and both *Clostridium* cluster XIVa (Fig. 4, Table 4) and Bacteroidetes (Fig. 5, Table 4). No trend was observed between methane emissions and *Clostridium* cluster IV (Table 4). Protozoa showed the strongest link with methane emissions among the various groups quantified (Fig. 6, Table 4), methane emissions increasing as protozoal abundance increased.

Table 2 | Methane production by cattle fed either or high- or medium-concentrate diets

Diet	High-concentrate		Medium-concentrate			Significance		
Breed	AA	Lim	AA	Lim	SED	Breed	Diet	Breed × Diet
Methane								
l/day	234	208	332	298	17.7	*	***	NS
g/day	152	135	216	194	11.5	*	***	NS
g/kg DM intake	13.5	13.6	21.3	22.3	1.14	NS	***	NS
kJ/MJ GE intake	39.0	39.9	61.7	64.2	3.31	NS	***	NS

***, P < 0.001;
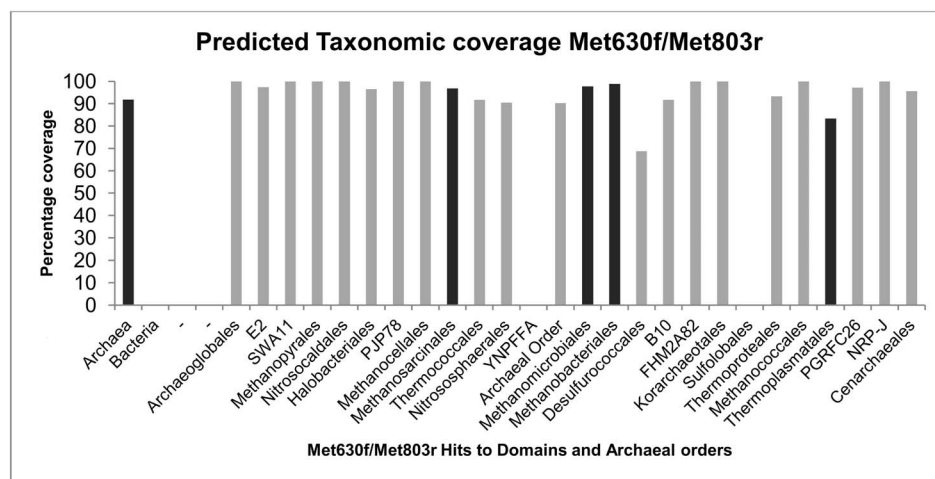*, P < 0.05.
DM – Dry matter; GE – Gross energy.

Figure 2 | Predicted domain coverage and specificity of archaeal primers. Dark bars are Methanogenic Archaea reported in the rumen. The coverage and specificity of Met630f and Met803r primers were determined *in silico* using PrimerProspector[33]. The primers were predicted to give >90% coverage of Archaea based on 867 reference sequences contained in the Greengenes 16S rRNA database, with only <5% coverage of non-target groups (Bacteria – 34562 reference sequences). The most prevalent methanogens reported in the rumen belong to the genera *Methanobrevibacter* (order, *Methanobacteriales*)[9,13], *Methanobacterium* (order, *Methanobacteriales*)[9,13], *Methanomicrobium* (order, *Methanomicrobiales*)[9,13], *Methanimicrococcus* (order, *Methanosarcinales*)[9,13] and Thermoplasmata (order, *Thermoplasmatales*)[10],with occasional *Methanosarcina* (order, *Methanosarcinales*)[13]. Thus, full coverage of ruminal methanogens would be predicted for Met630f and Met803r.

The ratio of archaea : bacteria (A : B) varied almost five-fold in digesta taken from individual animals immediately on leaving the respiration chamber, and the correlation with methane emissions was greater than other single comparisons (Fig. 7). Splitting according to diet (Fig. 8) resulted in a highly significant regression coefficient for the high-concentrate diet ($P < 0.001$), but no significant correlation for the medium-concentrate diet ($P > 0.05$).

Comparison of samples taken from live animals and *post mortem*. When the microbial community was compared in ruminal digesta samples taken by stomach tube from live animals immediately when they left the respiration chamber and from the same animals at slaughter, the abundance per ng DNA extracted was lower for all groups in both diets, except for archaea (Table 3). The proportions of the bacterial groups changed little, however (Table 3). The A : B ratio in *post-mortem* samples was similarly variable to the live-animal samples, and the ratio was also greater in samples taken from animals receiving the medium-concentrate diet (Fig. 9). The A : B ratios in the two types of sample were correlated (Fig. 9) with an overall correlation of R = 0.35, and R values within diet of 0.39. The correlation between methane emissions and A : B ratio was similar in

samples from live animals (R = 0.39) and *post mortem* (R = 0.38). As with the live-animal samples, splitting according to diet (Fig. 10) resulted in a highly significant regression coefficient for the high-concentrate diet ($P < 0.001$), but no significant correlation for the medium-concentrate diet ($P > 0.05$).

Multiple linear regression analysis of microbial abundance relative to methane emissions. Multiple regression models were fitted to predict CH_4 from microbial abundances in samples taken from live animals. The best model found, using Akaike's Information Criterion (AIC), included as predictors *Clostridium* cluster XIVa and archaea, and both terms were significant ($P < 0.001$) with $R^2 = 0.30$. These were independent effects when the other was accounted for. Including Bacteroidetes led to a marginally higher R^2, but it was not significant ($P = 0.22$). The prediction equation was:-

$$CH_4 = 17.20 + 0.0001453 \times Archaea - 0.0000464 \times IVa$$

Model selection was undertaken to obtain the best statistical model of the A : B ratio and its relationship to methane emissions,

Table 3 | Microbial numbers in samples of ruminal digesta taken from live animals and at slaughter (*p.m.*) (Means with SED for 24 observations per mean)

Diet	High-concentrate		Medium-concentrate			Probability		
Sample type	Live	*p.m.*	Live	*p.m.*	SED	Live/*p.m.*	Diet	L × D
X 10³								
Cluster XIVa[1]	125	72	69	52	8.7	<0.001	<0.001	0.003
Cluster IV[1]	131	58	158	82	14.8	<0.001	0.026	NS
Bacteroidetes[1]	255	143	179	88	14.3	<0.001	<0.001	NS
Total bacteria[1]	710	373	502	300	27.5	<0.001	<0.001	<0.001
Archaea[1]	28	31	42	57	4.2	<0.001	<0.001	0.022
Protozoa[2]	38	23	87	26	8.3	0.001	<0.001	<0.001
Proportion of total bacteria								
Cluster XIVa	0.177	0.185	0.140	0.174	0.013	0.013	NS	NS
Cluster IV	0.195	0.155	0.303	0.274	0.023	0.013	<0.001	NS
Bacteroidetes	0.370	0.394	0.359	0.287	0.024	NS	<0.001	0.004

[1]Results are expressed as copy number/ng DNA as determined by qPCR of 16S rRNA genes.
[2]Results are expressed as copy number/ng DNA as determined by qPCR of 18S rRNA genes.

Table 4	Correlation matrix (R) between methane emissions (g/kg DMI) and microbiota estimates (copy no/ng DNA) from live animals						
	Total bacteria	Cluster XIVa	Cluster IV	Bacteroidetes	Archaea	Protozoa	Methane
Total bacteria	1.000						
Cluster XIVa	0.637	1.000					
Cluster IV	0.090	0.314	1.000				
Bacteroidetes	0.613	0.125	−0.145	1.000			
Archaea	−0.328	0.188	0.336	−0.403	1.000		
Protozoa	−0.497	−0.313	0.142	−0.317	0.420	1.000	
Methane	−0.473	−0.347	0.112	−0.374	0.385	0.450	1.000

relative to breed, diet and experimental variables (Table 5). Results were similar for live-animal samples and *post-mortem* samples. The overall analysis showed that up to 79% of the variation in methane emissions can be explained by the A : B ratio and the other factors.

Discussion

It seems intuitive that methane emissions should correspond to the number of archaea, the only methane-producing microorganisms, present in the rumen of individual ruminant animals, in the present case beef steers. The biomass yield of the archaea must be directly proportional to the methane produced, since, with minor possible exceptions such as alcohol utilization[6], methanogenesis is the only mechanism of ATP synthesis available to the archaea. Furthermore, the cytochrome-containing genera[12] have not been reported in the rumen[13], so the molar growth yield of the different genera that are found in the rumen is likely to be similar. Nevertheless, repeated literature reports[4,14–16] and much anecdotal evidence have failed to find such a correlation, except when well known inhibitory chemicals such as bromochloromethane[17] and chloroform[18] have been used. Here, the relatively large number of animals used and the discrimination between breeds and diets have enabled us to find such a correlation, which was strongest when expressed as a ratio of bacterial abundance (A : B). Combined with the finding that *post-mortem* samples are as valid for this prediction as samples taken from live animals, a useful proxy has been established. Different micro-biomes in individual animals seem likely to be driven by the host animal[19], and the evidence suggests that differences in methanogenic archaeal communities in individuals probably persist over time[20], both of which factors probably played a part in our observations.

Broad groups of the rumen microbial community were quantified by small-subunit rRNA gene qPCR. A possibility that archaeal primers might not be adequate to include the newly defined order, Methanoplasmatales[10,11], was proved not to be of concern as a result of the good coverage of the primers used here, including the Thermoplasmatales order, in the *in silico* test. Generally speaking, the abundances of the various groups were consistent with those found previously. The Firmicutes, of which *Clostridium* groups IV and XIVa represent the majority found in the rumen, were as abundant or more abundant than the Bacteroidetes, as found by many others[21,22]. The Firmicutes were more abundant in the medium-concentrate (forage-containing) diet, in keeping with their role in fiber breakdown, in which the Bacteroidetes play a secondary role[22,23]. *Clostridium* Cluster IV, encompassing the highly cellulolytic *Ruminococcus* and several *Eubacterium* spp.[23] were more abundant with the medium-concentrate (forage-containing) diet. The Cluster XIVa grouping, whose abundance was lower in the medium-concentrate diet, would contain *Butyrivibrio* and related spp.[24], none of which is known to possess the ability to break down crystalline cellulose[23]. The archaea comprised 4% of bacteria in the high-concentrate diet and 8% in the medium-concentrate diet, compared to values of 2% in steers[22], 6% in reindeer[25] and 4% in a combined study of sheep, red deer and dairy cattle[26]. Protozoal abundance varied inversely with bacterial abundance. Ciliate protozoa were more numerous with the medium-concentrate diet, a result which seems to be at odds with the general observation that adding concentrate to a forage diet usually increases protozoal numbers[27]. However, the high-concentrate diet was an extreme diet, in which ruminal acidic conditions may have affected protozoa more than the positive effect of increased available starch for fermentation and growth.

In terms of our focus on CH_4 emissions, it was perhaps surprising that the H_2-producing *Ruminococcus* spp. of Cluster IV and total protozoa – which produce abundant H_2[27] - were not more correlated
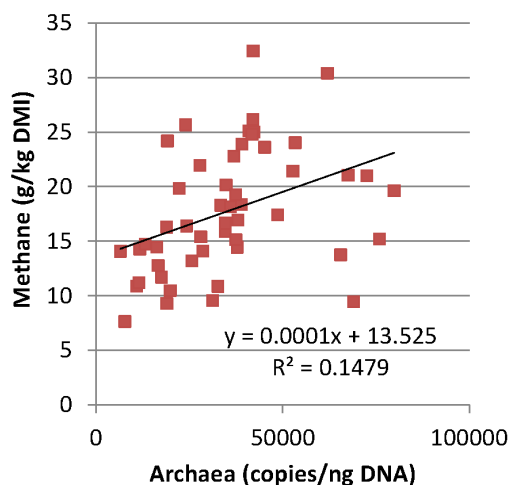

Figure 3 | Methane emissions and abundance of ruminal archaea in samples taken from live animals immediately after exiting the respiration chamber.
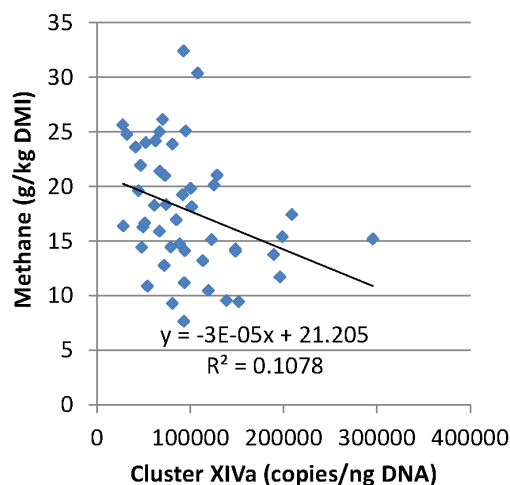

Figure 4 | Methane emissions and abundance of ruminal *Clostridium* Cluster XIVa in samples taken from live animals immediately after exiting the respiration chamber.
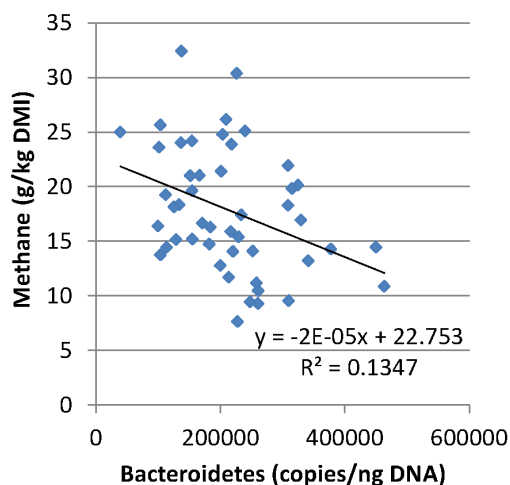
Figure 5 | Methane emissions and abundance of ruminal Bacteroidetes in samples taken from live animals immediately after exiting the respiration chamber.

Figure 6 | Methane emissions and abundance of ruminal ciliate protozoa in samples taken from live animals immediately after exiting the respiration chamber.

with CH_4, as H_2 is the main substrate for methanogenesis in the rumen[2–5]. It is possible that rates of H_2 production vary according to bacterial or protozoal species, and that a more detailed taxonomic description within the groups, best derived from metagenomic information, and phenotypic measurements of H_2 production might identify key genera and species that dictate H_2 production and thereby influence methanogenesis.

Protozoal, and to a lesser extent bacterial, numbers were clearly lower in all *post-mortem* samples, although archaea were changed little. The animals were not starved before slaughter, so a different feed intake immediately before feeding was not the cause. The cause must have been the different ways digesta were taken, by stomach tube in live animals compared to manual collection *post mortem*. Crucially, as indicated from all the analyses reported here, the archaea were unchanged between *in vivo* and *post mortem* sampling.

Cattle breed had no influence on methane emissions when corrected for differences in feed intake between breeds. Only minor differences in the microbial community were observed, as reported elsewhere[28].

One of the aims of these experiments was to investigate if a single proxy measurement might be useful as a predictor of methane emissions, irrespective of cattle breed or diet. The simplest proxy to emerge was the A : B ratio. The correlation did not reach significance with the medium-concentrate diet, but it was strong within the high-concentrate diet. More corroboration is required between diets, breeds and species, therefore. It may also be possible to refine the prediction using other measurements, such as transcriptomics of archaeal genes or chemical analysis of archaea-specific molecules like archaeol. The novel observation of possibly greatest usefulness is that the A : B ratio in *post-mortem* digesta corresponded well to the A : B ratio from the corresponding animals when they were alive. Thus, ruminal digesta sampling in the commercial abattoir, where breed and diet are often not known, followed by qPCR of extracted DNA for the enumeration of archaea and bacteria may be a useful tool in the campaign to lower methane emissions from livestock production. The method is inexpensive, it avoids the need for surgically modified animals to obtain digesta, it avoids the often cumbersome methods for measuring methane emissions, and it probably predicts methane emissions nearly as well as some more elaborate direct-measurement technologies. It will be particularly useful for large cohort sets that had previously been difficult to assess. Such cohort sets may be invaluable for assessing the genetic component of ruminant methane emissions.
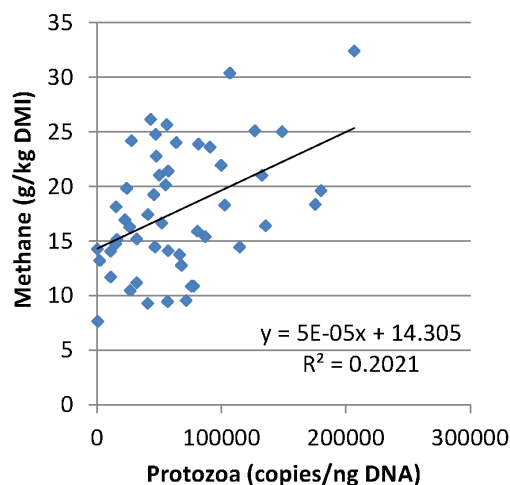
Methods

This study was conducted at the Beef Research Centre of SRUC (6 miles south of Edinburgh, UK) in summer 2011. The experiment was approved by the Animal Experiment Committee of SRUC and was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986.

Animals, experimental design and diets. The steers (n = 72) used in the experiment were from a rotational cross between Aberdeen Angus and Limousin sires, and are referred to as Aberdeen Angus (Limousin) crossbred animal depending on the breed of the purebred sire used. They were fed two complete diets *via* a forage wagon consisting (g/kg DM basis) of either 480 forage: 520 concentrate (medium-concentrate diet) or 75 forage: 925 concentrate (high-concentrate diet), respectively. The composition of the diets and nutritional composition of the feeding stuffs are given in Table 1. The steers were on the diet for a minimum of 8 weeks before methane measurements were made. One measurement of daily methane emission was carried out as described below.

Respiration chamber design, operation, and measurement. Six indirect open-circuit respiration chambers were used (No Pollution Industrial Systems Ltd., Edinburgh, UK). The chambers were fabricated from insulated panels with a window area of 12.7 m² and an internal floor area of 25.4 m² covered with cushioned anti-slip flooring (Stablesoft-Europe Ltd, Wellington, Somerset, UK). Within the chambers, steers were loose-housed in internal pens of 4 × 3 m. Consumption of feed was monitored from weigh cells located in feed bins and recorded at 10 s intervals. The chambers had access doors at the front for feeding and the rear for animal access and cleaning. The total volume (76 m³) was ventilated by recirculating fans set at 450 l/s. Air was removed from the chambers by exhaust fans set at 50 l/s. Thus there were approximately 2.5 air changes/h. Temperature and humidity control were achieved with air-conditioning units set at 15 ± 1°C and 60 ± 5% relative humidity,
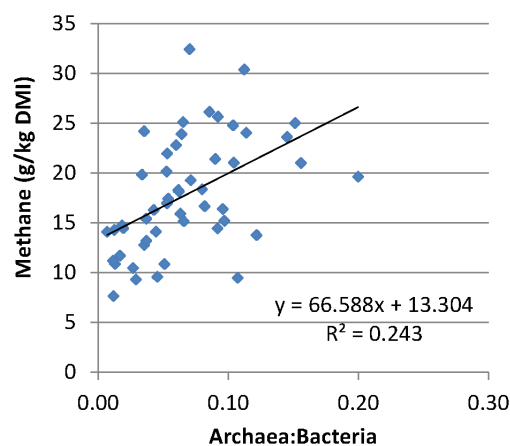

Figure 7 | Methane emissions and the archaea : bacteria ratio (A : B) in ruminal digesta samples taken from live animals immediately after exiting the respiration chamber.
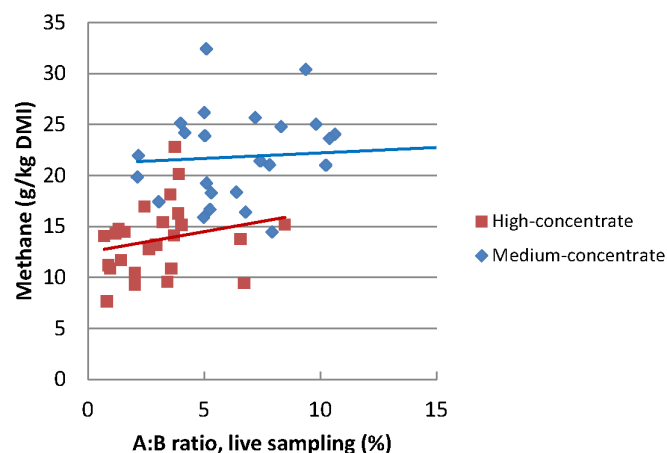
Figure 8 | Comparison of methane emissions of steers in respiration chambers and the archaea : bacteria ratio (A : B) in ruminal digesta samples taken from live animals, split according to diet.

Figure 9 | Comparison of the archaea : bacteria ratio (A : B) in ruminal digesta samples taken from live animals and at slaughter.

respectively. Chambers were operated under negative pressure (50 N/m²). The exhaust fan removed air immediately prior to re-entry of recirculated air and atmospheric air (ducted from outside the building) into the air-conditioning unit. Recirculated air was returned to the chambers at multiple points at the front of the chambers and removed from the back of the chambers again via multiple points. Total air flow was measured by in-line hot wire anemometers which were validated by daily measurements made with an externally calibrated anemometer (Testo 417, Testo Ltd, Alton, Hampshire, UK). Temperature and humidity were measured using sensor probes in the exhaust air outlet (Johnson Controls, Milan, Italy) and atmospheric pressure with a Vantage Pro2 weather station (Davis Instruments, Haywood, Ca, USA) and corrected for altitude.

Sampling points for exhaust air for gas analysis were located immediately after in-line hot wire anemometers. Inlet air was also sampled for measurement of ambient gas concentrations. Methane concentrations were measured by infrared absorption (MGA3000, Analytical Development Co. Ltd., Hoddesdon, UK). The analyser was calibrated with a gas mixture of known composition. Gas was sampled sequentially for 45 s from each chamber (including ambient air measurements) and the final stable value at 45 s recorded, thus giving 10 measurements per chamber per h. For 45 s before gas from each chamber entered the analyser, gas was pumped from the chamber (10 l/min) and vented from the system immediately prior to the analyser inlet to ensure there was no dead space in the system. Prior to the beginning of the experiment, gas recoveries were measured by releasing CO_2 at a constant rate into each chamber. The mean recovery was 0.98 (s.e. of mean, 0.030) and not different ($P > 0.05$) from 1.0.

In order to accustom the steers to the chamber environment, six days prior to chamber measurements groups of steers were moved to single pens of identical design to the pens within the chambers and within the same building as the chambers. Animals were then moved to the chambers and remained there for 72 h, with CH_4 and H_2 measurements recorded in the final 48 h being used for further analysis. Steers were fed once daily. Front doors of chambers were briefly opened at about 08.00 h daily to remove feed bins and again to replace bins with fresh feed at approximately 09.00 h. The pens were cleaned daily between 08.00 and 09.00 h with access by the rear doors. Exact times were recorded and doors opened in such a way as to minimise perturbation of the chamber environment.

Feed analysis. Feed samples were analyzed for DM, ash, CP, ADF and NDF according to (Ministry of Agriculture Fisheries and Food, 1992)[29]. Gross energy of feeds was performed on dried samples by adiabatic bomb calorimetry.

Digesta sampling and storage. Samples of digesta were taken from the steers immediately after they left the respiration chamber, using a tube (16 × 2700 mm) introduced into the esophagus *via* a nostril, then passed down to the rumen. Approximately 50 ml of digesta were immediately strained through two layers of gauze. Five ml of the strained ruminal fluid were mixed with 10 ml glycerol/phosphate buffered saline (30% v/v) and stored at −20°C. *Post-mortem* samples were taken within 30 min of slaughter. The rumen was opened and 50 g of digesta were taken from the dorsal sac. The samples were strained, diluted in glycerol/phosphate buffered saline and stored at −20°C.

DNA extraction was carried out using a method based on repeated bead beating plus column filtration[30]. Bacterial 16S rRNA genes were analyzed by qPCR using a BioRad iQ5. Archaea were amplified using the universal archaeal primers Met630F and Met803R[8]. Universal bacterial primers were UniF and UniR[31]. Bacterial 16S rRNA genes were analyzed by qPCR using a BioRad iQ5 and primers described by Ramirez-Farias et al.[24], where original citations may be found. The *Bacteroides* and *Prevotella* genera were quantified by Bac303F and Bfr-Fmrev: although Ramirez-Farias et al.[24] designed primers to quantify human *Bacteroides*, the genus *Prevotella*
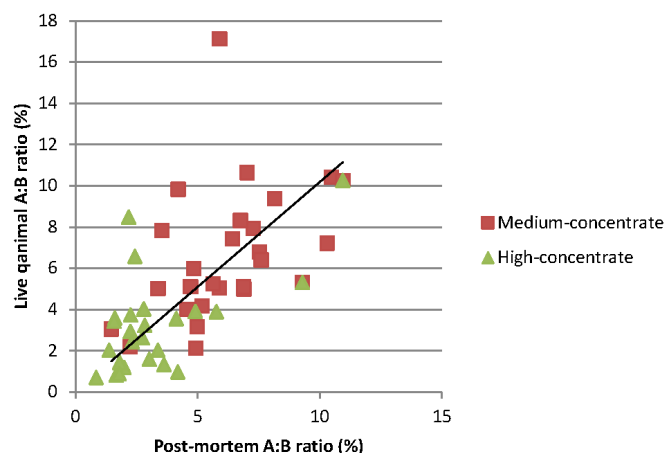
was also detected. *Clostridium* Cluster IV was quantified by primers Clep866mF and Clept1240mR and Cluster XIVa by primers Erec482F and Erec870R. Protozoal 18S rRNA gene numbers were also quantified by qPCR using primers described by Sylvester et al.[32]

Verification of qPCR primers. The universal archaeal primers Met630F and Met803R were verified *in silico* using PrimerProspector[33]. Each of the primers was aligned to the Greengenes 16S rRNA database[34] and putative amplicons generated. Taxonomic assignment was carried out using the RDP classifier[35] to assess the amplicons for coverage and specificity for the target group.

Calculations and statistical analysis. To minimize bias caused by entry of air when doors were opened for feeding and because during this period steers did not have access to feed, gas concentrations measured during this period were not used for further analysis and instead replaced by the mean value of measurements ($n = 10$) made in the last hour before doors were opened. If there was evidence that a steer had consumed food during that period, mean values for the hour preceding feed consumption were used.

All data from the chambers, that is gas concentrations, air flow, temperature, humidity and atmospheric pressure and records for feed consumption, were loaded into a database. Dry air flow was calculated and corrected to standard temperature and pressure for each individual record of gas concentration. Daily gas production was then calculated as the average of individual values (gas concentration × air flow) and converted to a mass basis.

One Limousin animal from the medium-concentrate diet was removed from the experiment due to illness, and three further animals were removed due to technical phenotyping issues, two Limousins on the high-concentrate diet and one Aberdeen Angus on the medium-concentrate diet. Data were analyzed using Genstat (Version 11.1 for Windows, VSN Int. Ltd., Oxford, UK), using linear mixed models where the factors were the 2 × arrangement of breed and diet and block and chamber. Data were reported as means and standard error of difference unless otherwise stated. The model selection analysis to develop the best statistical model was carried using the
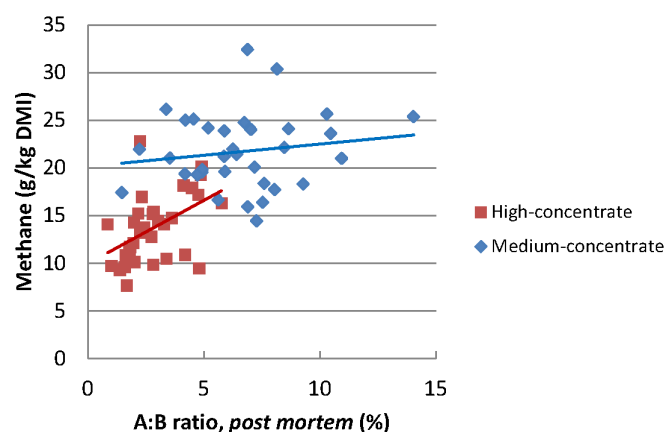

Figure 10 | Comparison of methane emissions of steers in respiration chambers and the archaea : bacteria ratio (A : B) in ruminal digesta samples taken from steers at slaughter, split according to diet.

Table 5 | Development of the best model for prediction of methane emissions from archaea : bacteria ratio based on rumen samples taken from live animals and rumen samples taken *post-mortem* 1 to 3 weeks after the chamber period, including breed

Prediction	Rumen samples taken	*P*-values of the effects tested in the models[a]							
		A : B ratio	A : B within Diet	Diet	Breed	Randomised block	Chamber	R²	RMSE[c]
CH₄ g/day	Live animals	0.0461	n.t.[b]	0.0003	0.1040	0.0043	0.1530	0.677	32.83
CH₄ g/day	Live animals	n.t.[b]	0.0250	0.0005	0.0911	0.0128	0.0540	0.705	31.78
CH₄ g/kg DMI	Live animals	0.0142	n.t.[b]	<.0001	0.2726	0.0015	0.0122	0.771	3.070
CH₄ g/kg DMI	Live animals	n.t.[b]	0.0089	<.0001	0.2632	0.0038	0.0031	0.791	2.970
CH₄ g/day	Post mortem	0.0007	n.t.[b]	0.0015	0.0035	0.0013	0.0732	0.672	30.45
CH₄ g/day	Post mortem	n.t.[b]	<.0001	<.0001	0.0019	0.0152	0.0151	0.725	28.10
CH₄ g/kg DMI	Post mortem	0.0150	n.t.[b]	<.0001	0.6694	0.0057	0.0263	0.720	3.155
CH₄ g/kg DMI	Post mortem	n.t.[b]	0.0049	<.0001	0.6379	0.0264	0.0072	0.743	3.048

[a]A fixed effect model with class effects of diet, breed, randomised block, chamber and as covariable the A : B ratio (alternatively across or within diets) was fitted.
[b]n.t. = not tested in this model because an alternative effect was fitted.
[c]RMSE is the root mean square error of the used model.

GLM procedure of SAS (Version 9.1 for Windows, SAS Institute Inc., Cary, NC, USA). Multiple linear regression models were fitted to predict CH₄ emissions from the whole dataset. Fitted terms included *Clostridium* Cluster IV, XIVa, *Bacteroides + Prevotella*, archaea and protozoa. To help with variable selection, all subsets of predictors were examined, with subsets compared using adjusted R-squared and Akaike's Information Criterion (AIC).

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Author contributions

A.W., R.J.W. and R.R. initiated the research. C.-A.D., J.J.H., D.W.R. and J.A.R. facilitated and carried out the animal work. N.M. and S.M.deS. performed qPCR. T.J.S. carried out bioinformatics associated with Supplementary Figures 1 and 2. R.J.W. wrote the manuscript. All authors provided feedback on the manuscript.

Additional information