animal

Methane emissions from beef cattle grazing on semi-natural upland and improved lowland grasslands

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In ruminants, methane (CH_4) is a by-product of digestion and contributes significantly to the greenhouse gas emissions attributed to agriculture. Grazed grass is a relatively cheap and nutritious feed but herbage species and nutritional quality vary between pastures, with management, land type and season all potentially impacting on animal performance and CH_4 production. The objective of this study was to evaluate performance and compare CH_4 emissions from cattle of dairy and beef origin grazing two grassland ecosystems: lowland improved grassland (LG) and upland semi-natural grassland (UG). Forty-eight spring-born beef cattle (24 Holstein–Friesian steers, 14 Charolais crossbred steers and 10 Charolais crossbred heifers of 407 (s.d. 29), 469 (s.d. 36) and 422 (s.d. 50) kg BW, respectively), were distributed across two balanced groups that grazed the UG and LG sites from 1 June to 29 September at stocking rates (number of animals per hectare) of 1.4 and 6.7, respectively. Methane emissions and feed dry matter (DM) intake were estimated by the SF_6 tracer and n-alkane techniques, respectively, and BW was recorded across three experimental periods that reflected the progression of the grazing season. Overall, cattle grazed on UG had significantly lower (P < 0.001) mean daily DM intake (8.68 v. 9.55 kg/day), CH_4 emissions (176 v. 202 g/day) and BW gain (BWG; 0.73 v. 1.08 kg/day) than the cattle grazed on LG but there was no difference (P > 0.05) in CH_4 emissions per unit of feed intake when expressed either on a DM basis (20.7 and 21.6 g CH_4 per kg DM intake for UG and LG, respectively) or as a percentage of the gross energy intake (6.0% v. 6.5% for UG and LG, respectively). However, cattle grazing UG had significantly (P < 0.001) greater mean daily CH_4 emissions than those grazing LG when expressed relative to BWG (261 v. 197 g CH_4/kg, respectively). The greater DM intake and BWG of cattle grazing LG than UG reflected the poorer nutritive value of the UG grassland. Although absolute rates of CH_4 emissions (g/day) were lower from cattle grazing UG than LG, cattle grazing UG would be expected to take longer to reach an acceptable finishing weight, thereby potentially off-setting this apparent advantage. Methane emissions constitute an adverse environmental impact of grazing by cattle but the contribution of cattle to ecosystem management (i.e. promoting biodiversity) should also be considered when evaluating the usefulness of different breeds for grazing semi-natural or unimproved grassland.

Implications

A study comparing upland and lowland grazing by beef-origin and dairy-origin cattle showed that, as a consequence of lower dry matter intake and fewer animals stocked per hectare, the BW gain of cattle grazing upland grassland was only 15% of those grazing intensively managed lowland pasture, but with 25% more methane emitted per kg of BW produced. Policies that encourage greater use of upland grassland for beef production may be counter-productive, through sacrificing animal production potential and increasing greenhouse gas emissions per kg of product.

However, using dairy steers in upland grazing opportunities has potential to enhance ecosystem services.

Introduction

Methane (CH_4) emissions represent important losses of ingested dietary energy in ruminant livestock and have been studied in considerable detail for well over 50 years, commencing with a decade of technically challenging and pioneering work at the Rowett Institute as summarised by Blaxter and Clapperton (1965), and added to by other research groups (Johnson and Johnson, 1995; Beauchemin and McGinn, 2006; Hart *et al.*, 2009; Clark, 2013). Methane

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losses account for between 2% and 12% of the gross energy (GE) intake of cattle, as dictated by the type, quality and quantity of feed consumed (Johnson *et al.*, 1994; Yan *et al.*, 2000; Yan *et al.*, 2009). More recently, the focus of interest in CH_4 has shifted towards its contribution to global greenhouse gas (GHG) emissions and, thence, to climate change (Clark, 2013). Agricultural systems (particularly those for milk and red meat production) produce ∼17% of overall global anthropogenic GHG emissions with cattle, alone, accounting for 15% of the CH_4 emissions (FAO, 2006). Clearly, this is of considerable importance as CH_4, when compared on an equal mass basis, is at least 20 times more potent than carbon dioxide as a GHG (Parry *et al.*, 2007).

Accurate CH_4 emission data for cattle and sheep are obtainable, albeit at some expense, using whole animal calorimeters (respiration chambers), in which the environment, feeding regime and animal behaviour are all tightly controlled. However, the appropriateness of extrapolating respiration chamber data to cattle grazing outdoors has, rightly, been questioned (Johnson *et al.*, 1994; Johnson and Johnson, 1995). The concern is especially relevant for marginal upland sites where topography, forage availability and its variable botanical and chemical composition, the energy cost of free-ranging foraging behaviour, grazing selection preferences, interactions between animals and fluctuating environmental conditions (warm *v.* cold; wet *v.* dry) all influence the quantity of CH_4 emitted by an animal.

Marginal land in temperate grassland regions (e.g. United Kingdom and Ireland) includes areas of upland semi-improved and semi-natural grassland. The productivity of semi-natural grasslands is constrained by factors such as climate, soil type, aspect and altitude but such areas are valued also, nationally and internationally, for their biodiversity. It is recognised that cattle have a role in the ecological management of these areas (Wright *et al.*, 2006; Dawson *et al.*, 2011). Anecdotally, traditional early-maturing breeds of cattle are better adapted to survive and utilise vegetation on the uplands because of their perceived hardiness relative to other beef breeds, but evidence for this is inconclusive (Rook *et al.*, 2004). Dairy-origin beef cattle are a significant 'by-product' of dairy herds and currently deliver 36% of the carcass output from the Northern Ireland beef industry (Titterington and Morrison, 2013) and these cattle could find use also as 'grazing agents' to maintain biodiversity in natural upland plant communities.

Typically, semi-natural grasslands are species rich (Pinares-Patino *et al.*, 2007) with an abundance of plant species with higher cell wall and lignin contents, and lower digestibility, than those species more commonly found in improved grassland (e.g. perennial ryegrass). This suggests that CH_4 production by cattle grazing these contrasting grassland types is likely to differ.

The United Kingdom Climate Change Act, 2008 set a target of reducing UK GHG emissions by at least 80% of 1990 levels (baseline) by 2050. To audit progress towards meeting this target, construction of a UK-wide inventory of GHG emission rates (the Agricultural UK GHG platform, 2013) was instigated by the UK Department for Environment, Food and Rural Affairs (Defra) and the governments of the United Kingdom devolved administrations. This inventory will cover a comprehensive range of ruminant production systems including both cattle (dairy and beef) and sheep. The development of the intraruminal SF_6 tracer technique by Johnson *et al.* (1994) provided a comparatively inexpensive method by which to estimate CH_4 emissions from a large number of grazing animals concurrently. The objective of the current study was to compare CH_4 emissions estimated using the SF_6 tracer technique, from beef cattle of different origins (dairy *v.* suckler) grazing on semi-natural upland grassland, with those from similar cattle grazing improved lowland grassland.

Material and methods

The study was conducted on an upland (UG) site at the College of Agriculture, Food and Rural Enterprise (CAFRE) Hill Farm, Glenwherry, Co Antrim, UK (54°50′N, 5°59′W; 312 m a.s.l.), and on a lowland (LG) site at the Agri-Food and Biosciences Institute (AFBI) farm at Hillsborough, Co. Down, UK (54°27′N, 6°4′W; 112 m a.s.l.). The study had the approval of the AFBI Hillsborough Ethical Review Committee.

Experimental sites and animals

The study involved concurrent measurements on similar groups of cattle at the two sites between June and August 2011. The UG site covered 16.7 ha of semi-natural and botanically diverse upland grassland consisting of native species such as *Holcus lanatus*, *Carex* spp., *Juncus effusus*, *Cynosurus cristatus*, *Agrostis capillaris*, *Deschampsia flexuosa*, *Phleum pratense*, *Anthoxanthum odoratum*, *Nardus stricta* and *Molinia caerulea*, together with patches of *Lolium perenne* and *Trifolium repens*. The LG site comprised 3.6 ha of predominantly perennial ryegrass (*L. perenne* L.). Each of the two sites was divided into four equal-sized paddocks of 4.2 ha (UG) and 0.9 ha (LG). Both sites received nitrogen (N) fertiliser applications in line with good agricultural practice (LG: 220 kg N/ha; UG: 30 kg N/ha).

Forty-eight spring-born beef cattle (year 2010), comprising 24 Holstein–Friesian (HF) steers, 14 Charolais-sired × Limousin × Holstein–Friesian steers (CHM) and 10 Charolais-sired × Limousin × Holstein–Friesian heifers (CHF) of mean initial BW 407 kg (s.d. 29), 475 kg (s.d. 36) and 422 kg (s.d. 50), respectively, were used in the study. The stocking rate (number of animals per hectare) at the UG and LG sites was 1.4 and 6.7, respectively. Cattle were balanced across the two sites (*n* = 24 per grassland type) according to BW, age, breed and gender and were then assigned across the four paddocks at each site, that is, two paddocks for HF (*n* = 6 per paddock), one paddock for CHM (*n* = 7) and one paddock for CHF (*n* = 5). Grazing commenced on 1 June 2011 and continued until 29 September 2011, during which there were three 12-day experimental periods: 20 June to

1 July (E1), 18 to 30 July (E2) and 15 to 27 August (E3). At the end of each period, cattle were re-allocated to another paddock, which they had not previously grazed. During each experimental period, the cattle were weighed on days 2, 3, 4, 9, 10, 11 and 12. A mean BW was calculated for each period and the BW gain (BWG) of each animal over the grazing season was calculated from the slope of the linear regression of BW against time.

The SF_6 tracer technique

Methane emissions were estimated using the SF_6 tracer technique developed by Johnson *et al.* (1994). Brass permeation tubes (45-mm long, 11-mm diameter and 71 mm^2 permeation surface area; P and T Precision Engineering Ltd, Naas, Ireland) were individually imprinted with a unique and indelible identification number. A circular Teflon$^{©}$ membrane (thickness: 0.27 mm) within the screw cap ensured containment of SF_6 while affording an essentially constant SF_6 release rate (Wills D. personal communication). The rate of SF_6 release from each filled permeation tube was determined, before ruminal insertion, by placing each tube in an incubator for 8 weeks at 39°C (to mimic intraruminal temperature) and weighing every 4 days (to 0.1 mg accuracy). The slope of the linear regression of weight against time was the SF_6 release rate (mg/day). Tubes with an R^2 of regression <0.995 were rejected. Acceptable tubes were allocated randomly to cattle (one per animal) and placed in the rumen on 26 May using a standard bolus dosing gun. The overall mean SF_6 release rate (all tubes) was 2.64 mg/day (s.d. 0.596). Mean release rates for UG and LG cattle were 2.642 (s.d. 0.567) and 2.635 (s.d. 0.636) mg/day, respectively.

Breath sampling

Exhaled and eructated gases were sampled at a point just above the animal's nostrils through use of a sampling tube connected to an evacuated 2.3-l plastic canister, suspended from a halter fitted to the animal's head (Supplementary Figure S1). In previous work at this Institute, a filter installed within the sampling line was identified as a cause of troublesome blockages in wet conditions and, accordingly, the nose piece arrangement used in the current study (Supplementary Figure S2) was modified from the original design of Johnson *et al.* (1994), and did not contain a filter.

Sample collection canisters were evacuated to −900 mbar using an oil-vacuum pump, at least 24 h before intended use. Each was tested for vacuum tightness using a Digitron 2022p digital manometer (RS Components, Corby, UK) before deployment. Canisters showing a significant loss of vacuum (>−870 mbar) were rejected. The rest were re-evacuated and their vacuum pressure recorded again, immediately before deployment. They were suspended from a halter on the animal's head to hang below the jawbone by ∼20 to 30 cm. The time of canister deployment was noted.

Sample flow to the vacuum canister was restricted to 0.35 to 0.45 ml/min by crimping a short section (∼40 mm) of 1/16″ × I.D. 0.004 stainless steel tubing (Alltech, Hillsborough, UK) installed within the sampling line. Flow rate was checked

during and after crimping using a Model Rz 32908-51 gas flow meter (Cole-Parmer Instruments Ltd, London, UK) with a flow measurement range of 0.1 to 1.0 ml/min. The crimped capillary also helped ensure that collection canister vacuums remained above half of their initial pressure (−900 mbar) until replaced 24 h later.

For each animal, gaseous emissions (diluted in surrounding air) were sampled from days 1 to 4 and from days 8 to 12 of each experimental period. Ambient CH_4 and SF_6 concentrations were measured in air samples captured in two evacuated canisters installed at each site on an elevated scaffold ∼20 m upwind from the paddocks (to minimise contamination by emissions from the experimental animals). These 'ambient' canisters also were replaced every 24 h. At disconnection, the pressure remaining in a canister, and the time of disconnection, were recorded. Canisters that did not have a residual pressure between − 750 and − 300 mbar were treated as suspect and the sampling equipment was checked for blockages or leaks. Patent canisters were pressurised with N to ∼500 mbar, the end pressure recorded and the contents analysed for SF_6 and CH_4 concentrations within 36 h.

Canister contents were analysed using a Varian 3800 gas chromatograph fitted with a Varian 200 6-port valve and connected to a Varian 1041 auto-injector (all from JVA Analytical, Dublin, Ireland). The instrument was calibrated weekly against three gas standards; low (10 ppt SF_6 and 10 ppm CH_4), medium (150 ppt SF_6 and 100 ppm CH_4) and high (300 ppt SF_6 and 300 ppm CH_4) supplied by Scott-Marrin Inc. (Riverside, CA, USA). The medium standard was run as an additional calibration check at the beginning and end of each day of measurement. At the laboratory, a sample from the canisters was directly injected through a 1-ml sample loop at a flow rate of 30 ml/min, with N as carrier gas, to determine the CH_4 and SF_6 concentrations simultaneously. This process was and replicate values were deemed acceptable if within a 3% tolerance limit. The CH_4 analysis column was a 1.2 m length and 2 mm i.d. stainless steel Porapak N 80-100 mesh column (Varian Inc., Walnut Creek, CA, USA) operated at 70°C. The SF_6 analysis column (Varian Inc.) was 1.8 m length × 2 mm i.d. and contained a 5 Å (45 to 60 mesh) molecular sieve. It was operated at 120°C. Methane was detected using a flame ionisation detector (FID) maintained at 250°C, whereas SF_6 was detected by an electron capture detector set at 300°C (both JVA Analytical). Account was taken of the N dilution factor in the canisters, and CH_4 (ppm) and SF_6 (ppt) were converted to $\mu g/m^3$ and CH_4 emissions (g/day) per animal were calculated as below (Lassey, 2013):

$$CH_4 \, (g/day) = (CH_4C - CH_4B)/(SF_6C - SF_6B) \times SF_6Q \\ \times MW \, CH_4/MW \, SF_6$$

where $CH_4C = CH_4$ concentration in the sample canister ($\mu g/m^3$); $SF_6C = SF_6$ concentration in the sample canister ($\mu g/m^3$); $CH_4B = CH_4$ concentration in ambient canister ($\mu g/m^3$); $SF_6B = SF_6$ concentration in ambient canister ($\mu g/m^3$);

$SF_6Q = SF_6$ release rate from permeation tube (g/min); MW CH_4 = molecular weight of CH_4; MW SF_6 = molecular weight of SF_6.

Feed intake measurement
Dry matter (DM) intake (DMI) was estimated for each animal during each experimental period using the *n*-alkane technique essentially as described by Mayes *et al.* (1986) but with alkanes dosed only once daily (Richmond *et al.*, 2014). During days 1 to 11 of each experimental period, all 48 animals were intraruminally dosed once daily with two 500 mg C_{32} alkane impregnated paper boluses.

Alkane bolus preparation
Boluses were prepared in batches of 50 before commencement of the study. Briefly, 50 g *n*-dotriacontane (C_{32}; Minakem Corp., Beuvry-la-Forêt, France) were dissolved in 450 ml of warmed (60°C) heptane and 5 ml portions of the solution were quickly pipetted into each of 50 pre-dried Rotilabo® cellucotton stoppers measuring 18 mm (bottom) × 22 mm (top) × 32 mm length (Karl Roth GmbH, Karlsruhe, Germany). During the dispensing process, the *n*-alkane solution was kept warm. Treated boluses were left upright in a fume hood for ~30 min before being placed in an oven at 100°C for 20 min, to ensure maximal dispersion of the *n*-alkane throughout each bolus and the removal of residual heptane. Five boluses from each freshly prepared batch were taken for quality assurance (QA) testing using the same preparation and test protocol as outlined for the herbage and faeces samples (see later). Mean bolus C_{32} content was 497 mg (s.d. 40.8).

Faecal sampling
A faeces sample (~200 g fresh weight) was taken *per rectum* from each animal, concurrently with administration of the *n*-alkane boluses, during days 8 to 12 of each experimental period. Faeces samples were stored at 4°C and later bulked to provide a 5-day composite sample for each animal, pending analysis for *n*-alkanes.

Vegetation sampling and analysis
Daily samples of vegetation (~200 g each and considered representative of the vegetation consumed, based on visual observation of grazing behaviour), were hand plucked from each paddock between days 5 and 9 of each experimental period for *n*-alkane analysis (Dove and Mayes, 2006) and for forage quality analysis. Some contamination of vegetation with C_{32} by faeces deposited during grazing is inevitable and is accepted as 'background'. However, to eliminate the risk of further contaminating vegetation samples with alkanes, staff involved in dosing C_{32} boluses and sampling of faeces were not permitted to also sample vegetation on the same day. GE concentrations (MJ/kg DM) were determined by isoperibol bomb calorimetry (Parr Instruments Co., Moline, IL, USA) according to Porter (1992). Nitrogen (N) concentration was determined by the high temperature Dumas combustion technique using a Vario MAX CN analyser (Elementar Analysensysteme GmbH, Hanau, Germany), and

CP concentration (g/kg DM) was then calculated as N concentration × 6.25. Ash (g/kg DM) was determined after combustion of samples at 550°C for 10 h. Vegetation DM concentration was determined by NIRS using a Foss 5000 scanner (Foss, Warrington, UK) through the AFBI Hillsborough Feeding Information System. Concentrations of NDF and ADF were determined as described by Cushnahan and Gordon (1995) using a Fibertec M 1020 hot extractor and 1021 cold extractor (Tecator AB, Hoganas, Sweden).

Determination of n-alkane concentrations
Vegetation and faeces samples were freeze-dried or oven-dried (60°C), respectively, and then hammer-milled to pass a 0.8-mm screen before saponification (at 70°C) and subsequent extraction with heptane at room temperature (modified from Dove and Mayes, 2006). The concentration of each alkane was determined in 0.5 µl of heptane extract by capillary gas chromatography (Dove and Mayes, 2006) on a Varian 3800 GC equipped with a Varian CP-8400 auto-sampler (both JVA Analytical) and ZB-1 (30 m, 0.25 mm i.d., 0.25-µ film thickness) coated capillary column (Phenomenex, Macclesfield, UK) and using a FID and 1079 split/splitless injector in split mode (split ratio 50 : 1), both maintained at 310°C. Helium was the carrier gas (1 ml/min constant flow rate) and the oven temperature was maintained at 290°C. The GLC was calibrated using a standard solution of synthetic C_{27} to C_{34} *n*-alkanes (Sigma-Aldrich, St Louis, MO, USA) to determine detector response factors. One in every 20 samples was an internal QA sample to ensure that the extraction and chromatographic procedures were thorough and quantitative.

Calculation of feed intake
Feed DMI (kg/day) was estimated using C_{32} and C_{33} *n*-alkane concentrations in the herbage (vegetation) and in faeces, according to the simplified equation of Mayes *et al.* (1986) below:

$$DMI\,(kg/day) = [D_{32} \times (FC_{32}/FC_{33})]/[HC_{33} - (FC_{32}/FC_{33}) \times HC_{32}]$$

where FC_{33} is the faecal C_{33} alkane concentration, FC_{32} the faecal C_{32} alkane concentration, HC_{33} the herbage C_{33} alkane concentration, HC_{32} the herbage C_{32} alkane concentration (all mg/kg DM) and D_{32} the dose of C_{32} alkane (mg/day).

Statistical analysis
All data relating to treatment effects (site and experimental period) for DM, ADF, NDF, GE, ash and CP were tested for normality before statistical analysis by REML repeated measures. Mean DMI and CH_4 were calculated for each animal during each period (E1 to E3). After passing a normality test, DMI, CH_4 and BWG data were statistically analysed using an auto-regressive model (order 1) in REML repeated measures. All data were also subjected to Fisher's Protected Least Significant Difference test. All statistical analyses were performed using GenStat 14.2 (VSN International Ltd, Hemel Hempstead, UK) for PC/Windows 7.

Results

Vegetation analysis

Concentrations of DM, ADF, NDF and GE, when averaged over all experimental periods (E1, E2 and E3) were significantly higher in UG than LG vegetation, whereas ash concentration was lower in UG than LG and CP concentration was similar in each (Table 1). Both ADF and NDF concentrations were lower in both UG and LG during E1 than in E2 or E3 while CP concentrations were lower in both UG and LG during E1 and E2 than in E3 (Table 1). There was no significant difference in DM or ash concentrations between experimental periods, and there were no significant interactions between site and period for any proximate constituent.

DM intake

Table 2 shows that estimated DMI (kg/day) when averaged over E1, E2 and E3 all experimental periods, was 10% higher from the LG than UG site. There were no significant differences or interactions in DMI ($P > 0.05$) between breeds, genders or experimental periods.

Methane emissions

The mean rate of CH_4 emission (g/day) was 15% higher at the LG than the UG site (Table 2), with mean emissions across both sites significantly ($P < 0.001$) lower during E1 than in the later periods (173, 196 and 198 g/day for E1, E2 and E3, respectively). There were no significant differences in CH_4 g/day ($P > 0.05$) between breeds or genders and there were no significant interactions. Mean within-animal coefficient of variation (CV) for CH_4 emissions was 20.3%, whereas between-animal variation in CH_4 emissions was greater for cattle grazing on UG than LG (30.8% and 24.2%, respectively).

Mean daily CH_4 emissions, expressed as g/kg DMI, were not significantly different between the UG and LG sites. Furthermore, there were no significant differences between experimental periods, genders or breeds and no significant interactions for these parameters. The between-animal CVs for CH_4 emissions (g/kg DMI) were 21.1% and 20.1% for cattle grazing the upland and lowland grassland sites, respectively. Figure 1 shows a strong linear relationship ($R^2 = 0.8711$) between mean DMI (kg/day) and mean CH_4 (g/day) emission when treating the mean values for both sites, and over all three experimental periods, as one dataset.

There was no significant difference ($P > 0.05$) between the UG and LG sites in the mean percentage of estimated daily GEI (MJ/day) lost as CH_4 (Table 2). Furthermore, there were no significant differences between experimental periods, genders or breeds, and no significant interactions for these parameters.

Table 1 *Mean proximate chemical composition of the upland (UG) and lowland (LG) vegetation for each experimental period (E1 – June; E2 – July and E3 – August)*

	Site		Period			Site		Period	
	UG	LG	E1	E2	E3	s.e.m.	Significance	s.e.m.	Significance
DM (g/kg fresh)	0.201	0.179	0.182	0.208	0.180	0.0501	**	0.0615	ns
ADF (g/kg DM)	259	238	230[a]	256[b]	259[b]	3.1	*	5.8	**
NDF (g/kg DM)	550	472	461[a]	523[b]	551[b]	9.2	***	11.2	***
GE (MJ/kg DM)	19.0	18.2	18.6	18.5	18.7	0.06	***	0.09	ns
Ash (g/kg DM)	63.2	89.1	70.8	79.5	78.1	4.18	***	3.94	ns
CP (g/kg DM)	161	169	153[a]	158[a]	184[b]	7.3	ns	8.9	*

DM = dry matter; GE = gross energy; ns = not significant.
[a,b]E1, E2 and E3 values within a row with different superscripts differ significantly at $P < 0.05$.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2 *Mean n-alkane estimated DMI (kg/day), SF_6 estimated methane (CH_4) emissions (g/day), CH_4 emissions as g/kg DMI, daily BW gain (BWG kg/day), daily CH_4 expressed as g/kg BWG and GEI g/day of cattle at both sites (UG and LG) over the experimental periods (E1 – June, E2 – July and E3 – August)*

	Site		Period			Site		Period	
	UG	LG	E1	E2	E3	s.e.m.	Significance	s.e.m.	Significance
DMI (kg/day)	8.68	9.55	8.90	9.21	9.24	0.139	***	0.173	ns
CH_4 (g/day)	176	202	173[a]	196[b]	198[b]	4.6	***	4.0	***
CH_4 (g/kg DMI)	20.7	21.6	20	21.4	22	0.61	ns	0.58	ns
BWG (kg/day)	0.73	1.08				0.037	***		
CH_4 (g/kg BWG)	261	197	211[a]	237[b]	239[b]	10.8	***	5.3	**
GEI (MJ/day)	6.02	6.48	5.93	6.29	6.52	0.182	ns	0.172	ns

DMI = dry matter intake; BWG = BW gain; GEI = gross energy intake; UG = upland pasture; LG = lowland pasture; ns = not significant.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
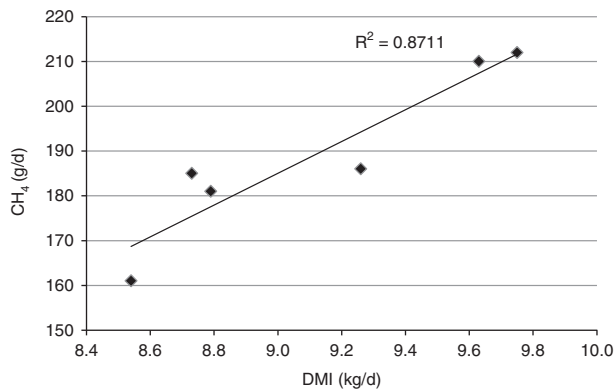
Figure 1 The relationship between mean *n*-alkane estimated dry matter intake (DMI; kg/day) and mean SF_6 estimated CH_4 emissions (g/day) by cattle grazing upland and lowland grasslands ($n = 24$ each) during all three experimental periods (June, July and August).

Animal performance and CH_4 emissions

The effect of grassland type on the BWG of cattle within each of the experimental periods could not be tested because BWG was calculated by linear regression of data across the grazing season (as changes in BW were determined only between, rather than within, experimental periods). Comparisons between sites are reported in Table 2, which shows that the mean BWG of animals at the UG site was 48% lower than that of their contemporaries at the LG site. There was no significant effect of breed on performance ($P > 0.05$) at either site. Mean daily CH_4 emissions expressed as g/kg BWG were 25% lower in cattle grazing the LG site than for those grazing the UG site (Table 2) but there was no significant breed difference ($P > 0.05$) in CH_4 emissions (expressed as g/kg BWG) at either site.

Discussion

Cattle that grazed on LG had greater daily DMI (by 10%), emitted more CH_4 per day (by 15%) and had greater BWG (by 48%) over the grazing season than counterparts grazing UG, but origin of cattle (dairy *v.* suckler) did not influence the measured traits.

The vegetation types identified in the current study differed in fibre content. Although the digestibility of the vegetation grazed at each site was not determined, it is well established that forage digestibility is inversely related to NDF concentration (Jung and Allen, 1995; Bruinenberg *et al.*, 2002) so that the greater NDF concentration in the upland vegetation suggests that its digestibility was lower than that of the more intensively managed lowland grassland throughout the grazing period. This is likely to have contributed to the lower DMI by cattle on upland vegetation (Jung and Allen, 1995; Beauchemin and McGinn, 2006; Decruyenaere *et al.*, 2009).

Non-animal factors known to affect grassland intake include site topography, location of water, sward structure and climatic conditions (Rook *et al.*, 2004; Decruyenaere

et al., 2009). In the current study, cattle on the UG site were observed to selectively graze predominantly grassy areas within the paddocks. This was reflected in the consistently low sward heights measured in these areas relative to other areas in which sedge, rush and upland grasses predominated (data not presented). Wallis de Vries and Daleboudt (1994) suggested that cattle are more likely to graze patches of short grass because of an inherently higher nutritional value. The contrast in animal performance between the LG and UG sites supports the predictions of Bruinenberg *et al.* (2002) that a beef animal, initially weighing 300 kg and consuming 6 kg DM/day, would gain 1.05 kg/day if grazing on perennial ryegrass but only 0.85 kg/day if grazing *H. lanatus/Agrostis* spp., and only 0.65 kg/day if grazing on *N. stricta*.

Methane emissions (g/day) were lower during E1 than during E2 or E3 but DMI did not differ between periods. However, over the entire grazing season, NDF and ADF concentrations were lower in E1 than in E2 or E3. Several studies have suggested that level of intake rather than digestibility is the main determinant of absolute CH_4 emissions (DeRamus *et al.*, 2003; Beauchemin and McGinn, 2006; Pinares-Patino *et al.*, 2007; Hart *et al.*, 2009; Pelve *et al.*, 2012; Boland *et al.*, 2013; Clark, 2013).

However, the difference in absolute CH_4 emissions between experimental periods in the current work cannot be explained solely by differences in DMI. McCaughey *et al.* (1999) found significant differences in DMI among cattle grazing swards of contrasting quality, but not between sampling periods, while corresponding absolute CH_4 emissions (L/day) differed significantly between sampling periods. In that study, differences in CH_4 emissions were explained by changes in nutritive value of the herbage. In contrast, Pinares-Patino *et al.* (2007) associated low CH_4 emissions by cattle grazing semi-natural grassland in spring with a lower DMI, but not with NDF intake. In a previous study, in which cattle grazed swards of Timothy of a range of digestibilities over a grazing season, intake of NDF (a partially digestible fraction of the cell wall) was identified as the most important factor in determining CH_4 production (Pinares-Patino *et al.*, 2003). In the current study, mean daily NDF intakes were 4.09 kg (E1), 4.78 kg (E2) and 5.08 kg (E3) across the two grassland types, following the trend in CH_4 (g/day) emissions. In the absence of determined values for NDF digestibility, no reliable conclusions can be drawn to explain the commonly observed lower CH_4 emissions in late spring and early summer.

Although significant treatment differences for CH_4 emissions and DMI did not follow the same pattern, CH_4 emissions per unit DM did not differ significantly between grassland types or experimental periods, suggesting that DMI is the primary determinant of CH_4 production (Figure 1), followed by quality factors. The absence of a significant difference in CH_4 emissions per unit DMI between cattle consuming vegetation differing in nutritive value supports this view. In a study of housed cattle fed herbage of either high or relatively low digestibility (Hart *et al.*, 2009), CH_4 emissions per kg DMI or energy lost in CH_4 as a proportion of GE intake did not differ
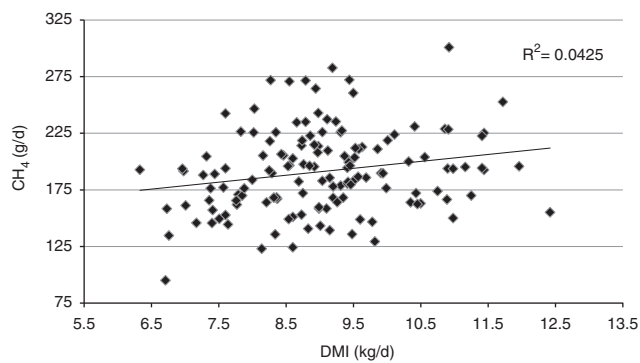
Figure 2 The relationship between *n*-alkane estimated dry matter intake (DMI; kg/day) and SF$_6$ estimated CH$_4$ emissions (g/day) for individual cattle grazing upland and lowland grassland ($n = 24$ per site) during all three experimental periods (June, July and August).

significantly between high and low quality grass (25.6 *v.* 25.7 g CH$_4$/kg DMI and 9.8% *v.* 9.9% GEI, respectively), although these authors had hypothesised that the lower digestibility herbage would emit more CH$_4$ relative to intake.

The CH$_4$ emission rates in the current study were lower than those reported by Hart *et al.* (2009) but lie within a range ($\sim$19.0 to 29.0 CH$_4$ g/kg of DMI) collated from an extensive range of other published work (McCaughey *et al.*, 1999; DeRamus *et al.,* 2003; Beauchemin and McGinn, 2006; Ellis *et al.*, 2007; Hart *et al.*, 2009; Pelve *et al.*, 2012); (Boland *et al.*, 2013; Clark, 2013; Jiao *et al.,* 2013). The percentage of GEI lost as CH$_4$ was also within the ranges commonly seen in the literature: 5.9% to 6.7% (Pinares-Patino *et al.*, 2003), 6.0% to 7.0% (Pinares-Patino *et al.*, 2007) and 5.6% to 6.1% (Boland *et al.*, 2013).

Within-animal and between-animal variation in CH$_4$ emissions is often greater when estimated by the SF$_6$ tracer technique than when measured in respiration chambers (McGinn *et al.*, 2006; Grainger *et al.*, 2007; Pinares-Patino and Clark, 2008). In the current study, between-animal variation in absolute CH$_4$ emissions was greater than within-animal variation, in line with several other studies, for example, Grainger *et al.* (2007) and Pinares-Patino and Clark (2008), but overall CVs of 30.8% and 24.2% (for emissions from animals grazing UG and LG, respectively) were greater than the range of 6.0% to 19.6% reported by others (McGinn *et al.*, 2006; Grainger *et al.*, 2007). The longer experimental period and the more variable diets used in the current study are likely to each have contributed to the higher CVs but when CH$_4$ emissions were expressed as g/kg DMI, the CVs were reduced to about 20% for cattle on each site, indicating that DMI is responsible for some of the variation in CH$_4$ emissions (Cottle *et al.*, 2011; Clark, 2013). However, the association between DMI (kg/day) and CH$_4$ emissions (g/day) for each animal in each period was poor (Figure 2; $R^2 = 0.043$). This contrasted with the strong relationship between mean DMI (kg/day) and mean CH$_4$ emission (g/day) over all three experimental periods (Figure 1) and suggests that other factors, such as variable weather conditions (wind, rain, etc.) may affect the efficiency of capture of breath

samples and thereby contribute to a greater variance in the determination of methane emissions by the SF$_6$ tracer technique.

The current study found no significant difference between the performance of dairy origin cattle (Holstein–Friesian steers) and suckler origin cattle (Charolais crossbred steers and heifers) on either the upland or lowland grasslands and lends support to the conclusions of Fraser *et al.* (2009) that pasture type has a greater impact than breed on beef cattle performance.

On a per unit area basis, the UG site produced only 15% of the BWG achieved from the LG site (128 *v.* 870 kg/ha, respectively), resulting in 25% greater CH$_4$ emission intensity from UG than LG. This apparent advantage of LG may be diminished when fuller account is taken of all environmental impacts and all ecosystem services and inputs. Intensive lowland systems require higher inputs than upland systems, such that the carbon footprint of their product is usually greater than from an upland system (Edward-Jones *et al.*, 2009). In any case, and irrespective of breed, cattle provide beneficial ecosystem services while grazing upland semi-natural vegetation, and this must also be taken into account (Wright *et al.*, 2006; Dawson *et al.*, 2011).

This study provides baseline data on CH$_4$ emissions from beef cattle grazing on upland grassland relevant to UK beef production systems (Agriculture UK GHG platform; Projects AC0114 and AC01115). The information provided will contribute to life cycle assessments of upland grazing scenarios on which judgements can be made, balancing sacrificed beef production, ecological benefits and contribution to emissions, all relative to lowland conditions.

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