**Enteric methane emissions and nitrogen utilisation efficiency for two genotype of hill
hoggets offered fresh, ensiled and pelleted ryegrass**

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Abstract

Thirty-six 12-month-old hill **hoggets** were used in a 2 genotype (18 Scottish Blackface vs. 18 Swaledale × Scottish Blackface) × 3 diet (**fresh vs. ensiled vs. pelleted ryegrass**) factorial design experiment to evaluate the effects of **hogget** genotype and forage type on enteric methane (CH₄) emissions and nitrogen (N) utilisation. The **hoggets** were offered 3 diets *ad libitum* with no concentrate supplementation in a single period study with 6 **hoggets** for each of the 6 genotype × diet combinations (n = 6). Fresh ryegrass was harvested daily **in the morning**. **Pelleted ryegrass was sourced from a commercial supplier (Aylescott Driers & Feeds, Burrington, UK) and the ryegrass silage was ensiled with Ecosyl (*Lactobacillus plantarum*, Volac International Limited, Hertfordshire, UK) as an additive**. The **hoggets** were housed in individual pens for at least 14 d before being transferred to individual respiration chambers for a further 4 d with feed intake, faeces and urine outputs and CH₄ emissions measured. There was no significant interaction between genotype and forage type on any parameter evaluated. Sheep offered **pelleted** grass had greater feed intake (e.g. DM, energy and N) but less energy and nutrient apparent digestibility (e.g. DM, N and neutral detergent fibre (NDF)) than those given fresh grass or grass silage ($P < 0.001$). Feeding **pelleted** grass, rather than fresh grass or grass silage, reduced enteric CH₄ emissions as a proportion of DM intake and gross energy (**GE**) intake ($P < 0.01$). Sheep offered fresh grass had a significantly lower acid detergent fibre (ADF) apparent digestibility, and CH₄ energy output (**CH₄-E**) as a proportion of **GE** intake than those offered grass silage ($P < 0.001$). There was no significant difference, in CH₄ emission rate or N utilisation efficiency when compared between Scottish Blackface and Swaledale × Scottish Blackface. Linear and multiple regression techniques were used to develop relationships between CH₄ emissions or N excretion and dietary and animal variables using data from sheep offered fresh ryegrass and grass silage.

The equation relating CH₄-E (MJ/d) to GE intake (GEI, MJ/d), energy apparent digestibility (DE/GE) and metabolisability (ME/GE) resulted in a high r^2 (CH₄-E = 0.074 GEI + 9.2 DE/GE – 10.2 ME/GE – 0.37, r^2 = 0.93). N intake (NI) was the best predictor for manure N excretion (Manure N = 0.66 NI + 0.96, r^2 = 0.85). The use of these relationships can potentially improve the precision and decrease the uncertainty in predicting CH₄ emissions and N excretion for sheep production systems managed under the current feeding conditions.

Keywords: methane production; nitrogen excretion; prediction equations; sheep

1. Introduction

Within the UK sheep production systems, approximately 43% breeding **hoggets** are hill breed types. The UK hill sheep sector is dominated by the Scottish Blackface (BF) breed with its outstanding qualities of survivability, adaptability and versatility. **Being excellent feeders, of strong constitution and lamb rearers, the Swaledale is a bold hardy sheep, well fitted to endure the hardships of exposed and high lying situations.** Research undertaken on hill farms around Northern Ireland has demonstrated that crossing BF ewes with Swaledale rams to produce crossbred **hoggets** can increase flock output compared with the pure BF. However there is limited data on the CH₄ emissions from crossbred hill **hoggets** in comparison to pure BF **hoggets**. CH₄ is produced in ruminants as an **unavoidable** by-product of enteric fermentation, a digestive process by which **nutrients** are broken down by micro-organisms into simple molecules for absorption into the bloodstream in the rumen. CH₄ is formed by CH₄-producing archaea, known as methanogens. Methanogens use a limited range of substrates, including CO₂/H₂, formate,

acetate, and methyl compounds. The type of carbohydrate fermented regulates the amount of H₂ available for CH₄ formation through the ratio of volatile fatty acids (VFAs), which are the major end products of carbohydrate digestion in the rumen (Johnson and Johnson, 1995). Increased structural carbohydrates in the diet shift the stoichiometry of the fermentation towards production of acetic and butyric acids, which leads to an increase in CH₄ production (Wilkerson et al., 1995). Increasing the proportion of non-structural in the diet lowers the amount of CH₄ produced, due to a shift in the fermentation pathways towards propionate, and a decline in the ruminal pH (Lovett et al., 2003). Pacheco et al. (2014) reported a range of 18.0 to 27.0 g CH₄ per kg dry matter intake from several experiments using respiration chambers to measure CH₄ emissions from sheep offered fresh perennial ryegrass. The Intergovernmental Panel on Climate Change (IPCC) Tier 2 methodology (IPCC, 2006), currently uses GEI, which is calculated from standard models, and a standard CH₄ conversion factor (CH₄-E/GE = 6.5%) to calculate CH₄ emissions. This conversion factor which was developed using data irrespective of the influence of different animal production levels and dietary factors is also applied to hill hoggets. Hill hoggets typically have lower body weights than lowland breeds. They are thus expected to have lower feed intakes theoretically and subsequently lower CH₄ emissions. It is also possible that physiological and/or behavioural differences between different breed types may lead to differences in amounts of CH₄ emitted. There is therefore an urgent need to develop more accurate CH₄ conversion factors specific to hill hoggets and representative of the breeds and rearing systems employed in the UK sheep production systems.

Another environmental concern for the sheep production systems is N excretion. Livestock urine and faeces are important components of the N cycle in pastures, where the microbial

processes in the soil produce nitrate and nitrous oxide which are responsible for groundwater pollution and global warming, respectively. Therefore, the accurate prediction of N output is essential for developing mitigation strategies to reduce the environmental impact of sheep production systems. However, only a few models to predict the N excretion have been reported in sheep offered legumes or foliages (Molle et al., 2009; Patra, 2010). Molle et al., (2009) recorded a range of 0.28 to 0.50 for faecal N/manure N in milked sheep grazing grass-legume binary mixtures differing in the legume component. Patra (2010) presented a mean value of 0.64 for faecal N/manure N in sheep of different breeds with foliages incorporated in the diets. These results may not be suitable for the grass-based diets in hill sheep due to the different diets and breeds. Most of the available CH₄ prediction equations are either based on lowland sheep data or on a collection of datasets from the literature without distinguishing the specific characteristics of hill sheep which are small and hardy breeds adapted to survive on low-quality forage in exposed upland conditions (Pelchen and Peters, 1998; Hammond, et al., 2013). The lack of such information can impact the development of appropriate mitigation strategies to reduce the environmental footprint for hill sheep production. Therefore, the objects of the present study were to investigate the effects of breed and forage types on enteric CH₄ emissions and the efficiency of N utilisation, and to use these data to develop prediction equations for CH₄ production and N excretion in hill hoggets.

2. Materials and methods

The present study was conducted under the regulations of Department of Health, Social Services and Public Safety of Northern Ireland in accordance with the Animals (Scientific Procedures) Act 1986 (Home Office, 1986).

2.1. Animals, experimental design, and diets

Thirty-six hill **hoggets** aged 12 months and weighed 42 ± 4.0 (mean $\pm$ SD) kg were used in a 2 genotype (18 Scottish Blackface and 18 Swaledale $\times$ Scottish Blackface) $\times$ 3 diet (fresh grass, **dried and pelleted** grass and grass silage) factorial design in the present study. Sheep within each genotype were offered 3 ryegrass only diets *ad libitum* in a single period study with 6 **hoggets** for each of the 6 genotype $\times$ diet combinations ($n = 6$): fresh grass, **dried and pelleted** grass and grass silage. **Dried and pelleted** grass was sourced from a commercial supplier (Aylescott Driers & Feeds, Burrington, UK) and pelletized from dried and ground perennial ryegrass. Fresh grass was harvested daily **in the morning** (from 02 May to 14 Jun, 2012) from a primary growth sward with perennial ryegrass, and the grass silage was made from the secondary growth (harvested on 30 June and 1 July, 2011) perennial ryegrass which was ensiled with Ecosyl (*Lactobacillus plantarum*, Volac International Limited, Hertfordshire, UK) as an additive. Deworming of the sheep was carried out before the beginning of the experiment using Cydectin (Zoetis, Surrey, UK). The 36 animals were individually housed at the same time in the commencement of the study for at least 14 d in pens before being transferred to individual respiration chambers for a further 4 d with feed intake, faecal and urine output and CH₄ emissions measured. The sheep were blocked in 6 groups in time with 6 animals per group balanced with genotype and diet to enter the 6 individual chambers with one sheep per chamber. The sheep were housed in

metabolic crates which were individually placed in chambers. Each crate contained a feed bin, drinking water container and separate trays to collect faeces and urine. The chambers were opened once daily at 0900 h to deliver diet and water and collect faeces and urine. The amount of feed offered was adjusted based on average feed intake of the previous two days to ensure a 10% refusal. The chemical composition of the experimental diets is shown in Table 1.

2.2. Measurements

Body weight (BW) was measured at the beginning of the study at the same time when the sheep were moved in the individual pens. Sheep were then gradually transferred to chambers in 6 groups with 6 animals in each group and weighted before entering and after leaving the chamber. Quantities of feed offered and refused were recorded daily during the experimental period for each animal, and samples of forages and refusals were retained daily for the measurement of DM content at 85°C for 24 h. During the final 4 d when animals were housed in chambers, the silage offered was sampled daily in two portions. One portion was bulked for every two days for determination of GE, N, pH, volatile fatty acids (VFA) and ammonia-N concentrations on a fresh basis. The other portion was dried at 85°C for 24 h daily and then bulked on a two-day basis for analysis of DM, NDF, ADF and ash. The DM of grass silage was then corrected as volatile corrected oven dry matter (VCODM) according to $VCODM = DM_{(85^{\circ}C)} + 0.716 \times (\text{Acetic Acid} + \text{Propionic Acid} + \text{n-Butyric Acid} + \text{i-Valeric Acid}) + 0.224 \times \text{Lactic Acid} + \text{NH}_3/\text{N} \times \text{N} + 0.97 \times (\text{Ethanol} + \text{Propanol})$ (Porter and Murray, 2001). Fresh grass samples were taken daily and dried at 85°C for 24 h and the dried samples were bulked on a two-day basis for analysis of GE, N,

NDF, ADF and ash. **Pelleted** grass was sampled daily and dried at 85°C for 24 h and the four-day samples were bulked for analysis of GE, N, NDF, ADF and ash.

The quantities of faeces and urine outputs were recorded daily during the 4 d in the respiration chambers. Urine samples were acidified during collection to a pH < 3.0 by addition of 2 M sulphuric acid. The faeces and urine samples taken during the first 3 d were stored at 4°C. Immediately after the last day of collection, the faeces and urine samples were pooled within animals, thoroughly mixed and representative samples were taken for analysis. The faeces samples were divided into two portions. One portion was used for measurement of N on a fresh basis immediately after the collection and the other portion was dried at 100°C for 48 h for determination of DM, and then milled (0.8 mm sieve size) for analysis of GE, NDF, ADF and ash. Urine sample was used for measurement of N and GE concentration, with GE measured in a 10 mL freeze-dried sample, which was contained in a self-sealing polythene bag of known weight and energy concentration. Manure N was calculated as the sum of faecal N and urine N.

Gross energy concentrations in forages, faeces and urine were determined in an isoperibol bomb calorimeter (Parr Instruments Co., Moline, Illinois, USA). Ammonia-N concentrations of silage samples were determined using methods described by Steen (1989). Total N concentrations were analyzed on a fresh basis for samples of silage, faeces and urine, and on a DM basis for fresh and **pelleted** grass samples using a Tecator Kjeldahl Auto 1030 Analyzer (Foss Tecator AB, Höganäs, Sweden) according to EU legislation No. 152/2009 Annex III. The concentrations of NDF and ADF were determined using the Tecator Fibretec System (Foss Tecator AB, Höganäs, Sweden) following procedures of Robertson and Van Soest (1981). Ash

was measured by combustion using a muffle furnace (Vecstar, Chesterfield, UK) at 550°C for 10 h (Method 942.05, AOAC, 1990). Silage pH was determined using a Metrohm 670 Titroprocessor fitted with an Orion Ross combination electrode. Digestible energy (DE) and metabolisable energy (ME) were based on the in vivo chamber data. DE was derived as the difference between GE intake and faecal energy. ME was calculated as the difference between GE intake and a sum of fecal energy, urine energy and CH₄ energy. Feeding level was calculated as ME intake divided by ME requirement for maintenance (ME_m) (AFRC, 1993).

Six indirect open-circuit respiration chambers were used in the present study. The 36 sheep were blocked in 6 groups in time with 6 animals per group balanced with genotype and diet to enter the 6 individual chambers with one sheep per chamber. Each group remained in the chambers for 4 d with measurement of CH₄ emissions. Methane values reported were the 4 d average for individual sheep. The respiration chambers were made with double Perspex walls and insulated fenestrated panels mounted on a profiled floor, incorporating airlocks for entry. The total volume of 5.4 m³ (2.0 m long, 1.5 m wide and 1.8 m high) was ventilated by suction pumps set at range of 16 – 20 m³/h, allowing a slight negative pressure within the chambers. Temperature and humidity control were achieved with air conditioning units set at 16 ± 1°C and 60 ± 10% relative humidity, respectively. The exhaust air removed from 6 chambers separately for measurement of volume, temperature, humidity and pressure. The 6 airflows were measured by 6 thermal mass flow meters (FCI ST50, Fluid Components International LLC, San Marcos, CA, USA) installed in the 6 ducts between each chamber and the gas analyser. The airflow meters were calibrated by a Testo 417 - Vane Anemometer (Testo AG, Lenzkirch, Germany) for each chamber. The CH₄ concentrations in the air into and out of each individual chamber were

measured every 14 minutes (the interval for each chamber set at 2 minutes) using a MGA3000 Multi-Gas Analyzer (ADC Gas Analysis Ltd, Hoddesdon, Herts, UK). The analyzer was calibrated weekly using oxygen free N₂ (zero gas) and a known quantity of CH₄ (span gas). This approach determined the absolute range (0–500 µL/L) and the linearity within this range. The flow measurement systems were checked before and immediately after the experiment by releasing analytical grade CH₄ into the chambers and by determining the recovery of CH₄. The purpose of the calibrations was to ensure a recovery rate of CH₄ at a range of 97 to 103%.

2.3. Statistical analysis

Data were analysed using two-way analysis of variance (ANOVA) as a 3 (diet) × 2 (genotype) factorial design for evaluation of the effects of genotype and forage type on feed intake, nutrient apparent digestibility, CH₄ emissions and N utilisation, with effect of initial BW removed, whereas 6 chamber measurement periods were fitted as block structure (the 36 animals were blocked in time in 6 groups). The linear and multiple regression techniques were also used to develop prediction equations for CH₄ output and N excretion using animal (i.e., BW, DM, energy and N intakes and feeding level) and dietary (i.e., energy digestibility and metabolisability, and OM and N content) factors, with data from sheep offered fresh ryegrass and grass silage only (n = 24) because of the significant effect of **pelleted** grass on feed intake, digestibility, CH₄ and N outputs. R² was the squared correlation of the response and the fitted values and represented the amount of variability explained. The significance of the explanatory variables fitted in the regressions was assessed using the Wald statistic. The regression procedure was sequentially adding terms to fixed model. Only the predictors with a significant effect ($P <$

0.05) on the relationship were left in the models. Akaike information coefficient (AIC) was also used to indicate the best fit. These relationships were developed using the restricted maximum likelihood model (REML): $Y = b + P + a_i X_i + e$, in which Y is the response variable; b is the regression intercept; P is the random effect of diet, genotype, and experimental period; a_i is the regression coefficient for explanatory factor i ; X_i is the effect of explanatory factor i ; and e is the random error. The statistical program used in the present study was Genstat (16th edition; Lawes Agricultural Trust, Rothamsted, UK) with a probability level of $P = 0.05$ for significance in treatments, interactions and relationships.

3. Results

There were no significant interactions between genotype and forage type on any variable of animal performance, nutrient apparent digestibility, enteric CH₄ emissions and nitrogen utilisation.

3.1. Animal performance and nutrient apparent digestibility

Sheep offered **pelleted** grass (Table 2) had greater DMI, DMI/BW, OM intake (OMI), NDF intake (NDFI), ADF intake (ADFI), GE intake (GEI), DE intake (DEI) and ME intake (MEI) ($P < 0.01$), but lower nutrient apparent digestibility of DM, OM, NDF, ADF, N and GE and digestible OM in DM (DOMD) ($P < 0.001$) than those given fresh grass and grass silage. There was no significant difference between fresh grass and grass silage on any variable of animal performance and nutrient apparent digestibility except that the ADF apparent digestibility of

grass silage was higher than that of fresh grass ($P < 0.001$). There was no significant difference in DMI, DMI/BW, GEI, DEI, MEI or FL between Scottish Blackface and Swaledale $\times$ Scottish Blackface. The apparent digestibility of DM, OM, NDF, ADF, N and GE and DOMD were not affected by the two genotypes either.

3.2. Enteric CH_4 emissions

Sheep offered pelleted grass (Table 3) had greater CH_4 emissions as g/day ($P < 0.01$) and as a proportion of BW ($P = 0.05$), but less CH_4 output as a proportion of DMI, GEI and OMI ($P < 0.001$) when compared to those given fresh grass and grass silage. The grass silage produced a significantly greater CH_4 -E as a proportion of GE than fresh and pelleted grass (0.067 vs. 0.057 and 0.044) ($P < 0.001$). CH_4 emissions as a proportion of digestible DM (DDM), digestible OM (DOM), DE and ME were similar among the three forage types. There was no significant difference between the two genotype of sheep for enteric CH_4 emissions.

3.3. Nitrogen utilisation

Sheep offered pelleted grass (Table 4) had greater N intake, faecal N and manure N output and faecal N/N intake ($P < 0.001$) and less urine N/N intake ($P < 0.05$) compared to those given fresh grass and grass silage. However, manure N/N intake or N retention/N intake was not affected by the diets. Nitrogen intake, output and utilisation were similar between fresh grass and grass silage. There was no significant difference between the two genotype of sheep for N intake, output and utilisation efficiency.

3.4. Prediction equations for CH₄ emissions

All relationships in the prediction equations for CH₄ emissions were significant ($P < 0.05$) and each predictor had a significant effect on the relationship ($P < 0.05$) (Table 5). CH₄ emissions were positively related to DMI, energy (GE, DE and ME) intake and BW when expressed as g/d or MJ/d, whereas negatively related to FL, DE/GE, ME/GE, ME/DE and GEI/metabolic BW (MBW) when expressed as a proportion of GE (i.e., CH₄-E/GEI). There were relatively strong relationships between CH₄ emissions and DMI (Eq. 3, $r^2 = 0.59$; standard error (SE) = 2.41) and between CH₄-E and GEI (Eq. 5, $r^2 = 0.56$; SE = 0.008), whereas the r^2 values were reduced and the SE values were increased when relating CH₄-E to MEI (Eq. 7, $r^2 = 0.50$; SE = 0.009) or CH₄ to BW (Eq. 1, $r^2 = 0.37$; SE = 5.18). Addition of ME/DE to the relationship between CH₄ and DMI considerably improved the r^2 and decreased the SE (Eq. 3 vs. 4, $r^2 = 0.59$ vs. 0.89; SE = 2.41 vs. 1.15). Similar improvement was also found in the relationship between CH₄-E and GEI after using DE/GE and ME/GE as supporting factors (Eq. 5 vs. 8, $r^2 = 0.56$ vs. 0.93; SE = 0.008 vs. 0.002). Dietary OM content and animal BW instead of DM intake were also used to predict CH₄ emissions because DM intake may not be available in commercial practice. The model performed satisfactorily and indicated CH₄ emissions were positively related to animal BW but negatively related to dietary OM content (Eq. 2).

3.5. Prediction equations for N excretion

All relationships in the prediction equations for N excretion were significant ($P < 0.05$) and each predictor had a significant effect on the relationship ($P < 0.05$) (Table 6). There was a strong relationship between N intake and manure N output (Eq. 14, $r^2 = 0.85$; SE = 1.39). Dietary N content and animal BW instead of N intake were also used to predict manure N excretion because N intake may not be available in commercial practice. The model performed satisfactorily and indicated both animal BW and dietary N content were positively related to manure N excretion (Eq.15). The current study also evaluated the effects of a number of dietary and animal factors on the efficiency of N utilisation in hill **hoggets** by relating, respectively, manure N excretion as a proportion of BW and N intake to a range of dietary and animal factors. Manure N excretion per unit of BW was positively related to N intake as a proportion of BW and GEI ($P < 0.05$). Manure N excretion as a proportion of N intake was negatively related to FL ($P < 0.01$).

4. Discussion

4.1. Prediction equations and mitigation strategies for enteric CH₄ emissions

Prediction equations are widely used to estimate CH₄ emissions from ruminants, due to the complex and expensive equipment required to determine CH₄ production *in vivo* (Yan et al., 2000). A range of prediction equations in sheep, beef and dairy cattle using DMI, GEI, MEI or BW as primary predictors for CH₄ emissions were developed, and found that DMI or GEI were better predictors than MEI or BW (Ellis et al., 2007; Yan et al., 2009; Jiao et al., 2013). The results obtained from the hill **hoggets** in the present study agreed with the previous reports.

However, the variation in CH₄ production predicted by DMI in the present study (0.59) is less than those reported in cattle (0.68 to 0.72) by Ellis et al. (2007), Jiao et al. (2013) and Yan et al. (2009), and that (0.85) reported in sheep by Hammond et al. (2013), possibly because of the relatively small dataset (24 sheep) in the present study.

These simple linear models, however, ignore the effects of diet nutrient compositions on rumen fermentation and CH₄ production. Equations that attempt to represent important aspects of diet characteristics and animal information, performed better in predicting CH₄ emissions than simple linear models (Hristov et al., 2013). Pelchen and Peters (1998) collected 1137 observations from sheep of different ages fed with different rations and developed the following regression equation: CH₄ (L/d) = 14.3 – 0.022 CP intake (g/d) + 0.0021 DEI (KJ/d) – 1.33 ME (KJ/g DM). Yan et al. (2009) found that using GEI together with energy apparent digestibility and ME/GE to predict CH₄ production resulted in a high r² value (0.84) in beef cattle: CH₄ (L/d) = (1.63 – 12.6 ME/GE + 11.3 DE/GE) × GEI (MJ/d) – 13.8. Moraes et al. (2014) identified gross energy intake, dietary fibre and lipid (EE) proportions, animal body weight and milk fat (MF) proportion as key explanatory variables for predicting emissions: CH₄ (MJ/d) = -9.3 + 0.042 GEI (MJ/d) + 0.094 NDF (%) – 0.38 EE (%) + 0.008 BW (kg) + 1.6 MF (%) based on a large database of North American dairy systems. The present study also found that the prediction accuracy could be improved using DMI or GEI as primary predictor together with DE/GE, ME/GE or ME/DE, rather than using DMI or GEI alone in a linear relationship (Eq. 3 vs. 4 and Eq. 5 vs. 8).

Increasing feed intake (expressed as feeding level in the present study) can reduce CH₄ losses as a proportion of DMI or GEI. In a comparison of the effect of forage types, McAllister et al. (1996) observed that when DM intake increased from 54.6 to 77.2 g/kg BW^{0.75}, CH₄-E output was reduced from 7.0% to 6.5% of GEI. In the present study, there was a negative relationship between feeding level and CH₄-E/GEI (Eq. 9) indicating that the percentage of dietary GE lost as CH₄-E reduced by 1.2 % per level of intake, which was greater than the values of 0.78% and 0.91% estimated by Yan et al. (2000, 2010) in cattle and lactating dairy cows but close to 1.3% measured by Hammond et al. (2013) in sheep and less than 1.6% reported by Johnson and Johnson (1995) in cattle. The negative relationships between feeding level and CH₄ production as a proportion of GEI are mainly derived from that CH₄ emission rate at maintenance level is higher than that at levels above maintenance. The dilution of maintenance is a major factor for high producing animals with a low rate of CH₄ emissions (Yan et al., 2010). In other words, to produce a given amount of product, increasing animal productivity can reduce total CH₄ emissions.

The multiple prediction equations obtained in the present study suggested that there was a considerable effect of diet nutrient composition on methanogenesis in the rumen of sheep. An increase in NDF concentration has been shown to increase CH₄ yield (g/kg DMI) in growing lambs offered fresh grass from perennial ryegrass and extensively managed permanent pasture (Fraser et al., 2015). The fresh grass and grass silage in the present study were both harvested from perennial ryegrass pasture. However, grass silage contained much higher NDF and ADF than fresh grass. The fermentation of cell wall carbohydrates results in a higher methanogenic progress in the rumen than the fermentation of non-cell wall components (Moe and Tyrrell,

1979; Johnson and Johnson, 1995). Thus, feeding fresh grass rather than grass silage, reduced CH₄ production per unit of GEI (5.7% vs. 6.7%) in the current study. Furthermore, negative relationships were observed between energy efficiency and CH₄-E as a proportion of gross energy intake. Increasing dietary DE/GE, ME/GE and ME/DE decreased CH₄-E/GEI (Eq. 10, 11 and 12). Yan et al. (2010) reported that CH₄-E/GEI was negatively related to energy metabolizability and ME/DE in lactating dairy cows. Pelchen and Peters (1998) found ME content was negatively relative to CH₄ emissions from sheep. Energy apparent digestibility or metabolizability normally depends on the quality of forage and the genetic merit of animal. The results therefore indicated that use of high quality feeds (e.g., feeds with high ME concentrations) and selection of animals with high energy utilisation efficiencies (e.g., animals with high genetic merit) offer effective approaches to reducing enteric CH₄ emission rates.

Pelleting forage can decrease CH₄ production. Blaxter and Clapperton (1965) showed that the reduction rate in CH₄-E/GE for each feeding level increase in sheep was higher for pelleted diets (2.1%) when compared to non-pelleted forages (0.8%). In the present study, pelleted grass increased DMI by 67.4% and 78.7% and reduced CH₄ production as a proportion of DMI by 21.3% and 25.7% when compared with fresh grass and grass silage, respectively. Increasing feed intake usually increases fractional passage rate in the rumen and decreases nutrient degradability (NRC, 2001). The rumen microorganisms therefore have less time available to ferment the feedstuff which would lead to increased ratio of propionate:acetate and thus reduce CH₄ production. Furthermore, a high passage rate can reduce Archaeal populations, leading to an accumulation of H₂ and a reduction in CH₄ emissions resulting from feedback inhibition on H₂ production (Janssen, 2010).

It is particularly important to develop more country-specific and accurate CH₄ emission data for countries/regions where ruminant production is a primary component of the whole economy. For example, the agriculture sector in Northern Ireland accounts for 30% of total GHG emissions, with 43.5% of agriculture contribution from livestock production, whereas the agriculture sector is a relatively minor contributor (9.8%) of GHG emissions within the whole UK (National Atmospheric Emissions Inventory (NAEI), 2014). However, the UK uses IPCC Tier 1 default emission factor (8 kg CH₄ head⁻¹ year⁻¹) (IPCC, 2006) for enteric fermentation for all mature sheep (NAEI, 2014). This may cause errors when developing CH₄ emission inventories for hill **hoggets** because the tier 1 emission factor does not consider the effects of diet and animal factors for CH₄ emissions (IPCC, 2006). The CH₄-E/GE obtained in the present study varied considerably which were 4.4%, 5.7% and 6.7% for **pelleted**, fresh and **ensiled** grass, respectively. CH₄-E/GE (5.7%) of the fresh ryegrass was close to that (5.4%) obtained in growing lambs fed fresh ryegrass and permanent pasture in Wales (Fraser et al., 2015), which is lower than the IPCC **Tier 2** value for sheep of 6.5%. Thus, CH₄ emissions from hill **hoggets** based on fresh ryegrass are likely to be lower than currently calculated using the default IPCC **Tier 2** methodology. The current data were derived from the local feed and sheep breed and can therefore be used to improve the precision and decrease the uncertainty in the development of CH₄ emission inventories for sheep production systems.

4.2. Prediction equations and mitigation strategies for manure N excretion

The present study examined the relationships between N excretion and a number of dietary and animal variables in hill **hoggets** and found that the best fitting factor was N intake. Using N intake as a single predictor for manure N output produced a high r^2 (0.85), which is comparable to those in sheep (0.86) (Patra, 2010), young Holstein cattle (0.86) (Jiao et al., 2014) and lactating dairy cows (0.90) (Yan et al., 2006). In situations in which N intake data are not available, prediction equations based on dietary N concentration and BW can produce a satisfactory assessment of manure N output (Eq. 15, $r^2 = 0.70$).

The prediction equations obtained in the present study and elsewhere indicate that manipulating dietary N concentration is an effective strategy to reduce N excretion. Manure N excretion per kg BW was positively related to N intake as a proportion of BW and GEI, respectively. These equations estimated that manure N output per kg BW could be increased proportionately by 0.61 and 0.23 g with an increase in N intake by 1 g per unit of BW and GEI, respectively. Molle et al. (2009) reported that N utilisation efficiency was negatively correlated with N intake and dietary N content in early-mid lactation sheep. Lactating goats have been reported to reduce urea N as a percentage of total urinary N from a range between 63% and 83% to 10% and 49% when changed from a diet adequate in N to a 27% N-reduced diet (Pfeffer et al., 2009). Milk N efficiency was greatest in the diet with the lowest forage crude protein in dairy ewes (Mikolayunas et al., 2011). These findings together with the results in the current study reflected that high dietary N concentration could result in the imbalance between nitrogen and energy supply to the rumen and excess degradable N above requirement which consequently cause additional N excretion and environmental impact.

On the other hand, increasing animal productivity is another mitigation approach to reduce manure N excretion per unit of production. In the current study, feeding level (as indicative of growth rate) was found to have a negative relationship with manure N excretion as a proportion of N intake. For example, the percentage of N intake lost as manure N could be reduced by proportionately 16% (Eq. 18) when increasing one level of feeding. Meanwhile, the percentage of dietary GE lost as CH₄-E could be reduced by 1.2% (Eq. 9) when increasing one level of feeding as discussed previously. This indicates that increasing feeding level could both decrease manure N excretion rate and CH₄ emission rate.

However, cautions should be taken when increasing the feeding level for some special diets. For example, in the present study, sheep offered dried **pelleted** grass had a much greater feed intake and a much less nutrient apparent digestibility (e.g., DM and N) than those offered grass silage and fresh grass, thus resulting in a much greater loss of OM and N in manure. The pollution potential of sheep manure is owing mainly to the characteristics of high organic load and significant amounts of N present in excreta (Orrico and Orrico, 2015). Therefore, measurements of total carbon footprint from sheep production systems in practice need to take account of emissions from not only enteric fermentation but also manure management and application. Furthermore, the energy required to produce grass silage (e.g. fossil fuels used for harvest and transportation) is likely to be higher than the energy required to produce grass. Similarly, the energy required to produce imported and pelleted feed (such as **pelleted** grass in the present study) is likely to be higher than the energy required to produce grass silage and fresh grass locally, resulting in additional CO₂ emissions during their manufacture and transportation to the animals. It is thus also important to take this into account when comparing the life cycle

assessment of GHG in production systems that use different feeding strategies, as this has been done for dairy systems (see for example O'Brien et al., 2012).

5. Conclusions

Feeding pelleted grass, rather than fresh grass or grass silage, increased feed intake, but reduced nutrient apparent digestibility, thus leading to a similar proportion of CH₄ emissions over digestible feed intake or N retention over N intake. There was no significant difference, in general, in CH₄ emission rate or N utilisation efficiency when compared between grass silage and fresh grass, or between Scottish Blackface and Swaledale × Scottish Blackface. A range of prediction equations for CH₄ production and manure N excretion were developed using diet and animal factors which were similar explanatory variables as those developed for other ruminant livestock but with specific coefficients for hill hoggets. Increasing feeding level and dietary OM content, and decreasing dietary N content, could optimise the mitigation of both CH₄ emissions and N excretion. These results add novel information for hill hoggets and can be used to predict CH₄ and N outputs and develop appropriate feeding and management strategies in reducing carbon footprint of sheep production systems.

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575 **Table 1.** Chemical composition of the experimental diets¹

Item	Pelleted Grass	Fresh Grass	Grass Silage
DM (g/kg)	893	203	312
Ash (g/kg DM)	64	67	87
CP (g/kg DM)	152	114	100
NDF (g/kg DM)	584	526	556
ADF (g/kg DM)	277	254	324
GE (MJ/kg DM)	19.0	18.4	18.1
ME (MJ/kg DM)	9.7	13.3	11.4
pH			3.7
NH ₃ -N /Total N (g/kg)			77.3
Acetic Acid (g/kg FM)			5.8
Propionic Acid (g/kg FM)			0.05
n-Butyric Acid (g/kg FM)			0.16
i-Valeric Acid (g/kg FM)			0.02
Lactic Acid (g/kg FM)			44.6

576 ¹ DM of Grass Silage was volatile corrected oven dry matter (VCODM); VCODM = DM_(85°C) +
577 0.716 × (Acetic Acid + Propionic Acid + n-Butyric Acid + i-Valeric Acid) + 0.224 × Lactic Acid
578 + NH₃/N × N + 0.97 × (Ethanol + Propanol) (Porter and Murray, 2001); FM = fresh matter
579 ME was based on the in vivo chamber data; ME = Gross energy – Faecal energy – Urine energy
580 – Methane energy. NDF and ADF were assayed without a heat stable amylase and expressed
581 inclusive of residual ash.

582

Table 2. Effects of forage type and genotype of hill **hoggets** on animal performance and nutrient apparent digestibility (n = 36)^{1, 2}

	Diet					Genotype			
	Pelleted	Fresh	Grass	SED	P-value	S×BF	BF	SED	P-value
	Grass	Grass	Silage						
Feed intake									
BW (kg)	49.0 ^b	43.0 ^a	42.7 ^a	1.72	0.001	42.9	47.0	1.43	0.007
DMI (kg/d)	1.59 ^b	0.95 ^a	0.89 ^a	0.106	<0.001	1.20	1.18	0.104	0.855
DMI/BW (g/kg)	32.8 ^b	22.1 ^a	20.8 ^a	2.30	<0.001	26.5	24.7	1.92	0.355
OMI (kg/d)	1.49 ^b	0.89 ^a	0.82 ^a	0.094	<0.001	1.08	1.09	0.078	0.972
NDFI (kg/d)	0.93 ^b	0.49 ^a	0.46 ^a	0.054	<0.001	0.64	0.64	0.045	0.947
ADFI (kg/d)	0.44 ^b	0.24 ^a	0.28 ^a	0.028	<0.001	0.32	0.33	0.024	0.879
GEI (MJ/d)	30.3 ^b	17.4 ^a	15.1 ^a	1.86	<0.001	21.2	21.5	1.55	0.894
DEI (MJ/d)	17.2 ^b	14.1 ^a	11.7 ^a	1.49	0.004	14.7	14.4	1.24	0.816
MEI (MJ/d)	15.5 ^b	12.7 ^a	10.3 ^a	1.39	0.004	13.2	12.8	1.16	0.767
Feeding level	2.05	2.07	1.64	0.229	0.147	2.03	1.85	0.191	0.352
Nutrient apparent digestibility									
DM (kg/kg)	0.59 ^a	0.82 ^b	0.81 ^b	0.019	<0.001	0.74	0.72	0.016	0.144
OM (kg/kg)	0.61 ^a	0.83 ^b	0.83 ^b	0.017	<0.001	0.76	0.74	0.014	0.113
DOMD (kg/kg)	0.57 ^a	0.77 ^b	0.75 ^b	0.016	<0.001	0.70	0.68	0.013	0.105
NDF (kg/kg)	0.55 ^a	0.80 ^b	0.83 ^b	0.021	<0.001	0.73	0.71	0.017	0.308
ADF (kg/kg)	0.51 ^a	0.80 ^b	0.87 ^c	0.022	<0.001	0.72	0.71	0.018	0.399
Nitrogen (kg/kg)	0.41 ^a	0.69 ^b	0.62 ^b	0.039	<0.001	0.58	0.56	0.033	0.476

GE (MJ/MJ)	0.57 ^a	0.80 ^b	0.77 ^b	0.020	<0.001	0.72	0.70	0.016	0.118
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¹ BF = Scottish Blackface; S×BF = Swaledale × Scottish Blackface; BW = average of BW

entering and BW leaving the chambers; DE and ME were based on the in vivo chamber data;

DE = Gross energy – Faecal energy; ME = Gross energy – Faecal energy – Urine energy –

Methane energy; DEI = DE intake; DOMD = digestible OM in DM; Feeding level = ME intake

divided by ME requirement for maintenance (AFRC, 1993); GEI = GE intake; MEI = ME

intake.

² a, b, c means within the same row with same superscripts are not significantly different ($P > 0.05$).

Table 3. Effects of forage type and genotype of hill **hoggets** on enteric methane emissions (n = 36)^{1, 2}

	Diet					Genotype			
	Pelleted	Fresh	Grass						
	Grass	Grass	Silage	SED	P-value	S×BF	BF	SED	P-value
CH ₄ (g/d)	24.0 ^b	17.8 ^a	17.3 ^a	1.90	0.002	20.3	19.5	1.58	0.587
CH ₄ /BW (g/kg)	0.49 ^b	0.42 ^a	0.40 ^a	0.038	0.050	0.47	0.41	0.032	0.109
CH ₄ /MBW (g/kg)	1.30 ^b	1.05 ^a	1.03 ^a	0.082	0.015	1.20	1.08	0.068	0.270
CH ₄ /DMI (g/kg)	15.0 ^a	19.1 ^b	20.3 ^b	1.33	0.001	18.6	17.5	1.11	0.304
CH ₄ /OMI (g/kg)	16.1 ^a	20.3 ^b	21.8 ^b	1.29	<0.001	19.8	18.8	1.08	0.332
CH ₄ /DDM (g/kg)	25.7	23.6	25.3	1.99	0.545	25.2	24.6	1.66	0.687
CH ₄ /DOM (g/kg)	26.5	24.6	26.5	1.80	0.484	26.1	25.6	1.50	0.761
CH ₄ -E/GE (MJ/MJ)	0.044 ^a	0.057 ^b	0.067 ^c	0.0039	<0.001	0.057	0.053	0.0033	0.202
CH ₄ -E/DE (MJ/MJ)	0.077	0.072	0.087	0.0061	0.060	0.080	0.077	0.0051	0.630
CH ₄ -E/ME (MJ/MJ)	0.086	0.081	0.100	0.0079	0.056	0.090	0.087	0.0066	0.704

¹ BF = Scottish Blackface; S×BF = Swaledale × Scottish Blackface; BW = average of BW

entering and BW leaving the chambers; DE and ME were based on the in vivo chamber data;

DE = Gross energy – Faecal energy; ME = Gross energy – Faecal energy – Urine energy –

Methane energy; MBW = metabolic BW (kg^{0.75}); DDM = digestible DM; DOM = digestible

OM; OMI = OM intake.

² a, b, c means within the same row with same superscripts are not significantly different ($P > 0.05$).

616 **Table 4.** Effects of forage type and genotype of hill **hoggets** on nitrogen utilisation (n = 36)^{1,2}

	Diet					Genotype			
	Pelleted	Fresh	Grass	SED	P-value	S×BF	BF	SED	P-value
	Grass	Grass	Silage						
N intake and output (g/d)									
N intake	38.7 ^b	17.4 ^a	14.2 ^a	2.21	<0.001	24.1	24.1	1.84	0.992
N intake/MBW (g/kg)	2.09 ^b	1.03 ^a	0.85 ^a	0.108	<0.001	1.41	1.31	0.090	0.707
Faecal N	23.0 ^b	4.9 ^a	5.3 ^a	1.44	<0.001	11.6	11.5	1.20	0.963
Urine N	10.3 ^b	7.8 ^{ab}	5.7 ^a	1.57	0.026	6.7	9.3	1.31	0.055
Manure N	33.3 ^b	12.7 ^a	11.0 ^a	2.02	<0.001	18.3	20.8	1.68	0.138
Retained N	5.4	4.7	3.2	1.72	0.452	5.8	3.3	1.43	0.086
Faecal N/MBW (g/kg)	1.24 ^b	0.30 ^a	0.32 ^a	0.077	<0.001	0.66	0.62	0.065	0.666
Urine N/MBW (g/kg)	0.56 ^b	0.45 ^{ab}	0.35 ^a	0.076	0.046	0.41	0.51	0.063	0.087
Retained N/MBW (g/kg)	0.29	0.28	0.17	0.087	0.559	0.33	0.18	0.073	0.115
N utilisation efficiency									
Faecal N/N intake	0.590 ^b	0.308 ^a	0.384 ^a	0.0393	<0.001	0.421	0.444	0.0328	0.476
Urine N/N intake	0.271 ^a	0.438 ^b	0.431 ^b	0.0680	0.031	0.340	0.407	0.0567	0.248
Manure N/N intake	0.861	0.746	0.815	0.0756	0.322	0.761	0.851	0.0631	0.161
Retained N/N intake	0.139	0.254	0.185	0.0756	0.322	0.239	0.149	0.0631	0.161

617 ¹ BF = Scottish Blackface; S×BF = Swaledale × Scottish Blackface; BW = average of BW

618 entering and BW leaving the chambers; MBW = metabolic BW (kg^{0.75})

619 ² a, b, c means within the same row with same superscripts are not significantly different ($P > 0.05$).

Table 5. Prediction equations for methane emissions from hill **hoggets** at age of 12 months (n = 24)^{1,2,3}

Equations	SE	r ²	AIC	Eq. No.
CH ₄ = 0.53 _(0.24) BW – 5.1 _(10.2)	5.18	0.37	135	(1)
= 0.40 _(0.23) BW – 43.8 _(18.87) OM + 41.3 _(22.5)	4.03	0.40	122	(2)
= 12.8 _(2.3) DMI + 5.7 _(2.2)	2.41	0.59	114	(3)
= 22.0 _(2.3) DMI – 125.6 _(22.8) ME/DE + 108.5 _(18.9)	1.15	0.89	91.6	(4)
CH ₄ -E = 0.040 _(0.008) GEI + 0.32 _(0.13)	0.008	0.56	0.12	(5)
= 0.042 _(0.008) DEI + 0.43 _(0.11)	0.008	0.55	0.67	(6)
= 0.042 _(0.009) MEI + 0.49 _(0.11)	0.009	0.50	2.49	(7)
= 0.074 _(0.007) GEI + 9.22 _(1.54) DE/GE – 10.2 _(1.48) ME/GE – 0.37 _(0.35)	0.002	0.93	-25.7	(8)
CH ₄ -E/GEI = -0.012 _(0.003) FL + 0.084 _(0.007)	0.00003	0.61	-121	(9)
= -0.092 _(0.040) DE/GE + 0.135 _(0.032)	0.00003	0.63	-120	(10)
= -0.12 _(0.031) ME/GE + 0.15 _(0.022)	0.00002	0.72	-126	(11)
= -0.47 _(0.04) ME/DE + 0.48 _(0.03)	0.000005	0.94	-158	(12)
= -0.031 _(0.008) GEI/MBW + 0.092 _(0.008)	0.00003	0.52	-125	(13)

¹ Values in subscript parentheses are SE; all relationships are significant ($P < 0.01$).

² AIC = Akaike information coefficient; BW = average of BW entering and BW leaving the chambers (kg); CH₄ = methane emissions (g/d); CH₄-E = CH₄ energy (MJ/d); DE and ME were based on the in vivo chamber data; DE = Gross energy – Faecal energy (MJ/kg DM); ME = Gross energy – Faecal energy – Urine energy – Methane energy (MJ/kg DM); DEI = DE intake (MJ/d); FL = feeding level as ME intake divided by ME requirement for maintenance (AFRC,

628 1993); GEI = GE intake (MJ/d); MBW = metabolic BW ($\text{kg}^{0.75}$); MEI = ME intake (MJ/d);

629 OM = organic matter content in diets (kg/kg DM)

630 ³ Only data from sheep offered fresh grass and grass silage were used (n = 24).

631

Table 6. Prediction equations for N excretion from hill **hoggets** at age of 12 months (n = 24)^{1, 2, 3},

g/d

Equations		SE	r ²	AIC	Eq. No.
Manure N	= 0.66 _(0.11) NI + 0.96 _(2.26)	1.39	0.85	118	(14)
	= 0.49 _(0.14) BW + 0.51 _(0.19) N – 18.2 _(6.1)	2.30	0.70	119	(15)
Manure N/BW	= 0.61 _(0.11) NI/BW + 0.041 _(0.051)	0.0006	0.82	-50.7	(16)
	= 0.23 _(0.08) NI/GEI + 0.041 _(0.086)	0.0014	0.56	-42.3	(17)
Manure N/NI	= -0.16 _(0.04) FL + 1.05 _(0.11)	0.003	0.90	-14.3	(18)

¹ Values in subscript parentheses are SE; all relationships are significant ($P < 0.01$).

² AIC = Akaike information coefficient; BW = average of BW entering and BW leaving the chambers (kg); FL = feeding level as ME intake divided by ME requirement for maintenance (AFRC, 1993); GEI = GE intake (MJ/d); N = nitrogen content in diets (g/kg DM); NI = N intake (g/d).

³ Only data from sheep offered fresh ryegrass and grass silage were used (n = 24).