



**Feed Into Lamb (FIL): an investigation of metabolisable
energy requirements and environmental footprint of sheep
toward developing a robust energy feeding system for
sustainable sheep production**

Final project report

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EXECUTIVE SUMMARY

The data used in this report were collated from 5 sheep calorimeter chamber experiments undertaken at AFBI from 2013 to 2017. These experiments were funded by the current project (trial codes: E44, E45 and E46) and the previous DEFRA project (trial codes: S63 and S64) AC0115 – Improvements to the National Inventory: Methane. The data collated were used to develop: 1, updated maintenance energy requirements for the current sheep flocks; 2, prediction equations for enteric methane emissions from sheep; 3, nitrogen utilization efficiency and prediction of nitrogen excretion in sheep. These results have been published in 6 leading international scientific journals and 15 scientific conference proceedings (References are presented in Appendix 2).

Development of updated maintenance energy requirements for the current sheep flocks

Energy intake and out data ($n = 131$) used were collated from 5 experiments with sheep (5 to 18 months old and 29.0 to 69.8 kg BW) undertaken at this Institute from 2013 to 2017. These data were analysed using the REML analysis to develop the linear relationship between energy balance (E_g) or heat production (HP) and ME intake, with the effects of a range of dietary and animal factors removed. The net energy (NE_m) and ME (ME_m) requirements for maintenance derived from the linear relationship between E_g and ME intake were 0.358 and 0.486 MJ/kg^{0.75}, respectively, which are 40% to 53% higher than those recommended in energy feeding systems currently used to ration sheep in the USA, France and the UK. Further analysis of the current dataset revealed that concentrate supplement, sire type or physiological stage had no significant effect on the derived NE_m values. However, female lambs had a significantly higher NE_m (0.352 vs. 0.306 or 0.288 MJ/kg^{0.75}) or ME_m (0.507 vs. 0.441 or 0.415 MJ/kg^{0.75}) than those for male or castrated lambs. The present results indicate that using present energy feeding systems in the UK developed over 40 years ago to ration the current sheep

flocks could underestimate maintenance energy requirements. There is an urgent need to update these systems to reflect the higher metabolic rates of the current sheep flocks.

Prediction of enteric methane emissions from sheep

The data used were collected from 82 sheep offered fresh perennial ryegrass (*Lolium perenne*) as sole diets. Sheep were from breeds of Highlander, Texel, Scottish Blackface and Swaledale, at age of 5 to 18 months, and weighting from 24.5 to 62.7 kg. These data were analysed using the restricted maximum likelihood procedure to develop prediction equations for CH₄ emissions. The mean CH₄ production was 21.1 g/kg DM intake or 0.062 MJ/MJ GE intake. Dry matter intake and GE intake were much more accurate predictors for CH₄ emissions than BW ($r^2 = 0.86$ and 0.87 vs. 0.09). Adding grass DE and ME concentrations and grass nutrient concentrations (e.g., OM, N, GE, NDF and WSC) to the relationships between DM intake or GE intake and CH₄ emissions improved prediction accuracy with R^2 values increased to 0.93 . Models based on farm level data, e.g., BW and grass nutrient (i.e. DM, GE, OM and N) concentrations were also developed and performed satisfactorily ($R^2 = 0.63$). These models can contribute to improve prediction accuracy for enteric CH₄ emissions from sheep grazing on ryegrass pasture.

Nitrogen utilization efficiency and prediction of nitrogen excretion in sheep

The data used were collected from 82 sheep offered fresh perennial ryegrass (*Lolium perenne*) as the sole diet. Sheep were from breeds of Highlander, Texel, Scottish Blackface and Swaledale, at age of 5 to 18 months, and weighting from 24.5 to 62.7 kg. These data were analysed using the linear mixed model procedure to develop prediction equations for faeces N, urine N and manure N. Nitrogen intake was the best single predictor for N output in faeces, urine and manure, and the r^2 value for prediction of manure N output was greater than those for faeces N and urine N (0.86 vs. 0.70 and 0.77 , respectively). Animal BW and herbage DM, ether extract, NDF, ADF, water soluble carbohydrate and DE concentrations and N digestibility, instead of N intake, were also used to predict N outputs because N intake may not be available in

commercial practice. The prediction equations for N utilization efficiency indicated that increasing feeding level and ME concentration and reducing N concentration could improve N utilization efficiency and shift N excretion into faeces rather than urine. The equations developed in the current study therefore provided an approach for sheep producers to quantify N excretion against production and consequently to develop their own mitigation strategies to reduce the environment impact from sheep production systems.

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Chapter 1.

Introduction

Nutrition is a key factor affecting the performance and economics of sheep flocks in Northern Ireland. For example, nutrition of ewes during pregnancy has been demonstrated to affect their health and fertility, as well as the growth performance of their lambs. The environmental impact of sheep systems, in terms of greenhouse gas emissions and nutrient deposition (nitrogen and phosphorus) is also closely linked to diet. Knowledge of the nutrient requirements of sheep is therefore important for promoting efficient and cost-effective use of feed resources, to promote high levels of performance and to reduce their environmental footprint.

Current feeding standards for breeding ewes in the UK are largely determined from the Technical Committee on Responses to Nutrients (TCORN) Report published by the Agriculture and Food Research Council (AFRC, 1993). In the 20 years since its publication, there have been no attempts to update or refine these recommendations. This is in contrast to the dairy sector where concerns over feeding recommendations, especially for high yielding dairy cows, were largely addressed by the Feed Into Milk (FiM) Project.

Several well-documented concerns over the UK feeding standards for sheep have been highlighted, particularly in relation to the prediction of metabolisable energy requirements for maintenance, and the contribution of fat mobilization to these requirements, particularly in the pregnant ewe. For example, previous AFBI studies demonstrated that AFRC recommendations for growing sheep underestimated metabolisable energy requirements for maintenance by up to 30%, as reported by Dawson and Steen (1998) in a modelling exercise of AFBI sheep chamber data, and by Yan and Xue (2009) in a literature review funded by Department for Environment,

Food and Rural Affairs. In light of these concerns, there is a need to investigate the maintenance energy requirements of sheep and to update current feeding standards accordingly, particularly for breeding ewes. There is also a need to better understand the feed requirements for growing replacement ewes.

The overall objectives of this project were: 1, to review and update the current UK feeding standards for sheep, in terms of their metabolisable energy requirements; and 2, to develop prediction equations for enteric methane emissions and manure (faeces and urine) nitrogen outputs for the current sheep flocks. Calorimetry studies were used to investigate metabolisable energy requirements for maintenance in growing and adult animals, and the effects of breed, sex/status, and nutrition status on both metabolisable energy requirements and the associated environmental footprint. These data were used to develop new improved models for prediction of the energy requirements of sheep toward developing a robust and precise energy feeding system for sustainable sheep production.

Chapter 2.

Updating maintenance energy requirement for the current sheep flocks

Abstract

The objectives of the present study were to develop updated maintenance energy requirements for the current sheep flocks and evaluate if these requirements were influenced by a range of dietary and animal factors. Data ($n = 131$) used were collated from 5 experiments with sheep (5 to 18 months old and 29.0 to 69.8 kg BW) undertaken at this Institute from 2013 to 2017. The trials were designed to evaluate the effects of dietary type, genotype, physiological stage and sex on nutrient utilization and energetic efficiencies. Energy intake and output data were measured in individual calorimeter chambers. Energy balance (E_g) was calculated as the difference between GE intake and a sum of faecal energy, urine energy, methane energy and heat production (**HP**). Data were analysed using the REML analysis to develop the linear relationship between E_g or HP and ME intake, with the effects of a range of dietary and animal factors removed. The net energy (NE_m) and ME (ME_m) requirements for maintenance derived from the linear relationship between E_g and ME intake were 0.358 and 0.486 MJ/kg^{0.75}, respectively, which are 40% to 53% higher than those recommended in energy feeding systems currently used to ration sheep in the USA, France and the UK. Further analysis of the current dataset revealed that concentrate supplement, sire type or physiological stage had no significant effect on the derived NE_m values. However, female lambs had a significantly higher NE_m (0.352 vs. 0.306 or 0.288 MJ/kg^{0.75}) or ME_m (0.507 vs. 0.441 or 0.415 MJ/kg^{0.75}) than those for male or castrated lambs. The present results indicate that using present energy feeding systems in the UK developed over 40 years ago to ration the current sheep flocks could underestimate maintenance energy requirements.

There is an urgent need to update these systems to reflect the higher metabolic rates of the current sheep flocks.

Introduction

The sheep industry in most of leading sheep production and exporting countries (e.g., New Zealand, Australia and Uruguay) has experienced a considerable change in production structure during the last 30 years, which reflects improved individual productivity and reduced sheep population (Montossi et al., 2013). In the UK, a sheep quota system was introduced in 1992 which imposes an upper limit to the number of sheep eligible for subsidy support payments (Conington et al., 2001). Such policies certainly require sheep industry to take actions to improve genetic traits of sheep flocks. There is evidence indicating that from the late 1990s, sheep genetic merit has increased by 4% per year in terms of productivity and product quality (Banks, 2003). The improvement in sheep genetic merit would certainly influence the basal metabolism. A range of previous studies suggested that current high genetic merit ruminants tended to have higher metabolic rates and require more energy for maintenance than those over 30 years ago (Costa et al., 2013; Jiao et al., 2015; Zou et al., 2016). Recent calorimetric studies showed that net energy (NE) requirement for maintenance (NE_m) for sheep ranged from 0.267 to 0.298 MJ/kg^{0.75} (Deng et al., 2014; Salah et al., 2014; Rodrigues et al., 2016) which are greater than those recommended by NRC (1985, 0.234 MJ/kg^{0.75}) and INRA (1989, 0.250 MJ/kg^{0.75}). Dawson and Steen (1998) found a much higher ME requirement for maintenance (ME_m) for growing lambs than that proposed by AFRC (1993). The above results imply that current sheep flocks with high genetic merit may have greater maintenance energy requirements than those recommended in current energy rationing systems. The maintenance energy requirements for sheep recommended by AFRC (1993), INRA (1989) and NRC (1985) were developed using data obtained over 40 years ago. There is an urgent need to update these systems to reflect the higher basal metabolic rates of the current sheep flocks. Therefore, the objectives of the present study were to address this knowledge gap by developing

updated maintenance energy requirements using recent sheep calorimeter chamber data and to evaluate the effects of a range of animal and dietary factors on energetic efficiencies for the current sheep flocks.

Material and methods

All procedures adopted in the present sheep experiments were approved by the Ethical Review Committee of the Agri-Food and Biosciences Institute (Hillsborough, UK) and were in accordance with the UK Animal Scientific Procedures Act (1986).

Animals, treatments and experimental procedure

The data ($n = 131$) used in the present study were collated from 5 sheep experiments undertaken in this Institute from 2013 to 2017. These trials were designed to evaluate the effects of concentrate supplement, genotype, physiological stage and lamb sex on feed intake, nutrient digestibility, energy and nitrogen utilization efficiencies and enteric methane emissions. Trial 1 used 48 growing lambs (5 months old and 36 ± 5.0 kg BW) in a factorial design study, with 2 sire genotypes (Highlander vs. Texel) \times 3 sexes (female vs. male vs. castrated) \times 2 dietary types (fresh grass vs. fresh grass plus 0.5 kg/d pelleted concentrate). Trial 2 used 16 replacement ewes (13 months old and 61.5 ± 5.3 kg BW) in a factorial design study with 2 sire genotypes (Highlander vs. Texel) \times 2 dietary types (fresh grass vs. fresh grass plus 0.5 kg/d pelleted concentrate). Trial 3 used 24 dry ewes (16 months old and 47.6 ± 5.1 kg BW) in a factorial design study with 2 sire genotypes (Belclare vs. Lleny) \times 3 feeding levels (1 feeding level vs. 1.5 feeding levels vs. ad libitum feeding). Trial 4 used 32 growing lambs (5 months old and 37.8 ± 3.2 kg BW) in a factorial design study with 2 sire genotypes (Meatline vs. Suffolk) \times 2 sexes (female vs. castrate) \times 2 dietary types (fresh grass vs. fresh grass plus 0.5 kg/d pelleted concentrate). Trial 5 used 16 replacement ewe lambs (8 months old and 35.6 ± 5.2 kg BW) in a factorial design with 2 sire genotypes (Lleny vs. Suffolk) \times 2 dietary types (grass silage vs. grass silage plus 0.5 kg/d pelleted concentrate).

Sheep were fed fresh grass in the first 4 trials and grass silage in the final trial. Fresh grass and grass silage used in the experiments were produced from predominantly perennial ryegrass (*Lolium perenne*) swards containing a range of varieties (e.g., *Aberstar*, *Aberzest*, *Fetione*, *Magella*, *Menna*, *Merbo*, *Merlinda* and *Spelga*). Fresh grass was harvested daily in the morning. The grass silage was produced from secondary growth material and ensiled with Ecosyl (*Lactobacillus plantarum*, Volac International Limited, Hertfordshire, UK) as an additive. The concentrates used in these experiments included a mineral-vitamin supplement and some of the following ingredients: cereal grains (maize, barley), by-products (maize gluten meal, wheat feed, soybean hull, sugar cane molasses, distillers dried grains with soluble) and protein supplements (soybean meal or rapeseed meal).

Calorimeter measurements

Energy intake and output data were measured using indirect open-circuit respiration calorimeter chambers. Before transferred to calorimeter chambers, all animals were individually housed in pens and offered experimental diets for at least 19 d with free access to water. Afterward, animals were transferred to individual calorimeter chambers and stayed there for 5 d with measurement of feed intake, faeces and urine outputs and gaseous exchange (methane, carbon dioxide and oxygen) in the final 4 d. The sheep were individually housed in metabolic crates, which were placed in individual chambers. The detailed description of equipment, sampling procedures, analytic methods and calculations used in the calorimetric studies were published by Zhao et al. (2015).

Statistical analysis

The ME intake was derived as the difference between GE intake and a sum of faecal energy, urine energy and methane energy. Energy balance (E_g) was calculated as the difference between ME intake and heat production (HP). The HP (MJ/d) was determined from measurements of oxygen consumption, carbon dioxide and methane emissions (L/d) and urinary nitrogen output (g/d) using the equation of Brouwer (1965).

All data were evaluated using the linear mixed regression technique by Genstat 16.2 (16th edition; Lawes Agricultural Trust, Rothamsted, UK). The data were fitted using the Eq. [i] and [ii]. The effects on these relationships by experiment, animal age, chamber number, dietary type, genotype, sex and physiological stage were used as random effects which were removed.

$$E_g = a_1 \text{ ME intake} + b_1 \quad [i]$$

$$HP = a_2 \text{ ME intake} + b_2 \quad [ii]$$

The unit for E_g , HP and ME intake is MJ/kg^{0.75}. The constant (b_1 or b_2) was taken as the NE_m (MJ/kg^{0.75}) and the corresponding ME_m (MJ/kg^{0.75}) was calculated from the constant divided by the slope (b_1/a_1) in Eq. [1].

Further analysis was undertaken to compare the effects of concentrate supplement, sire genotype, physiological stage and sex of lamb on maintenance energy requirements. These analyses were conducted by dividing the whole data, respectively, into a number of sub-datasets for each comparison, i.e., comparison of concentrate supplement (forage only diets (n = 75) vs. mixed diets of forage and concentrates (n = 56)), sire genotype (maternal sire (n = 59) vs. terminal sire (n = 72)), physiological stage (lamb (n = 96) vs. ewe (n = 35)) and lamb sex (male lamb (n = 16) vs. female lamb (n = 48) vs. castrated lamb (n = 32)). The linear relationship between E_g and ME intake was used to evaluate effects on maintenance energy requirements within each comparison, by comparing constants obtained between sub-datasets with a common

slope, or by comparing slopes with a common constant. Explanatory variable of each comparison was fitted as fixed effect, whereas factors of experiment, age of animal, chamber number and factors except for the one which was evaluated were treated as random effects in all models. For regressions obtained from each comparison, Fisher's least significant difference test was used to calculate the pair-wise differences between the different constants or different slopes, if the fixed effect was significant. Finally an assessment of the goodness-of-fit of each model was made by calculating a pseudo R^2 value.

Results

Animal, diets, and nutrients utilization

The data of animals, diets, and nutrient and energy utilization used in the present study are presented in Tables 1 and 2. The data represented a very wide range of BW (29.0 to 69.8 kg) and dietary forage proportion (0.448 to 1.000 kg/kg DM), CP (0.091 to 0.227 kg/kg DM) and fibre contents (ADF = 0.184 to 0.345 kg/kg DM and NDF = 0.382 to 0.544 kg/kg DM). Consequently, large differences were obtained in DMI, GE intake, faecal energy, urine energy, methane energy and HP. Mean ME intake was 14.3 MJ/d with a range from 5.6 to 27.7 MJ/d. Energy balance ranged from -4.6 to 15.9 MJ/d with a mean value of 4.7 MJ/d. The differences between maximum and minimum data for digestibility (kg/kg) of nitrogen, ADF, NDF, OM and digestible OM in DM were respectively 0.425, 0.263, 0.270, 0.162 and 0.217, and the corresponding data (MJ/ME) for DE/GE, ME/GE, HP/ME intake and E_g /ME intake were respectively 0.192, 0.201, 1.331 and 1.331.

Development of updated maintenance energy requirement

The linear regression equation between E_g or HP and ME intake using the whole data is presented in Table 3 and Fig. 1. These 2 relationships were highly significant ($P <$

0.001), with the R^2 values of 0.765 and 0.534, respectively. The NE_m value derived from the 2 equations was $0.358 \text{ MJ/kg}^{0.75}$ and the corresponding ME_m value was $0.486 \text{ MJ/kg}^{0.75}$ calculated assuming zero E_g .

Effects of dietary and animal factors on maintenance energy requirement

The effects of dietary and animal factors on maintenance energy requirements of sheep are presented in Table 4 and Fig. 2. The evaluation was undertaken by comparing the constants with a common slope within each comparison. All relationships were highly significant ($P < 0.001$), with R^2 values ranging from 0.764 to 0.807. The analysis found that concentrate supplement (forage diet vs. mixed diet), sire type (Maternal vs. Terminal) or physiological stage (lamb vs. ewe) had no significant effects on constants (i.e., NE_m or ME_m) within each comparison. However, within the comparison of lamb sex, female lambs had a significant higher constant than those for male and castrated lambs ($P < 0.045$).

A similar evaluation was also undertaken to evaluate the effects of these dietary and animal factors on slopes with a common constant within each comparison (Table 5). The similar results were obtained, i.e., concentrate supplement, sire type or physiological stage had no significant on the slopes within each comparison, while female lambs had a significant higher slope than those for male and castrated lambs ($P < 0.048$).

Discussion

The data used in the present study represented a broad range in terms of genotype, animal age, BW, plane of nutrition, concentrate supplement and forage type (grazed grass vs. grass silage). This dataset therefore covered a wide range of production conditions for the current sheep flocks in the UK.

Energy requirement for maintenance

Maintenance energy requirements recommended in energy feeding systems to ration sheep across the world are mainly derived from calorimeter data through fasting metabolism measurements or regression analysis techniques. The linear regression technique was also used in the present study to develop updated NE_m ($0.358 \text{ MJ/kg}^{0.75}$) and ME_m ($0.486 \text{ MJ/kg}^{0.75}$) for the current sheep flocks using data collated from 5 sheep calorimeter studies undertaken at this Institute from 2013 to 2017. The present NE_m or ME_m value is proportionately 53%, 43% and 40% higher than those currently recommended to ration sheep in the USA (NRC, 1985; $NE_m = 0.234 \text{ MJ/kg}^{0.75}$), France (INRA, 1989; $NE_m = 0.250 \text{ MJ/kg}^{0.75}$) and the UK (AFRC, 1993; $ME_m = 0.348 \text{ MJ/kg}^{0.75}$), respectively. The present ME_m values for male ($0.441 \text{ MJ/kg}^{0.75}$) and female ($0.507 \text{ MJ/kg}^{0.75}$) are higher than that recommended in Australia (SCA, 1990; $0.420 \text{ MJ/kg}^{0.75}$), while SCA (1990) proposes a higher value for castrated lambs than the present ME_m (0.471 vs. $0.415 \text{ MJ/kg}^{0.75}$). High ME_m values for sheep were also reported recently (0.418 to $0.433 \text{ MJ/kg}^{0.75}$) in a range of calorimeter chamber studies (Deng et al., 2014; Salah et al., 2014; Cárdenas et al., 2018) and in an early study (Dawson and Steen, 1998; $0.460 \text{ MJ/kg}^{0.75}$). The discrepancy between AFRC (1993) and the present study might be attributed to the fact that the maintenance energy requirement of AFRC (1993) was derived from fasting metabolism measurements of sheep after a long period of restricted feeding (usually at maintenance levels). Publications suggested that restricted feeding for a long period could lower basal metabolism and fasting HP of ruminants (Marston, 1948; Agnew and Yan, 2000). For example, Ferrell et al. (1986) showed that lambs on a high plane of nutrition had a higher fasting HP value by 40% than those on a low nutrition level prior to fasting. Furthermore, Chowdhury and Ørskov (1994) found that fasting after a lengthy period of restricted feeding could result in deamination of tissue amino acids for supply of glucose, thus likely inducing a number of metabolic disorders in ruminants. Therefore, Yan et al. (1997) suggested that it would be more appropriate to feed ruminants at production levels prior to fasting

when using fasting metabolism to estimate basal metabolic rates. Alternatively, animals can be given feeds to supply one-third of maintenance energy requirements, rather than fasting during measurement of the energetic efficiencies (Chowdhury and Ørskov, 1994).

On the other hand, Agnew and Yan (2000) attributed the higher maintenance energy requirements for the current ruminants, to their higher production efficiency and higher body lean (protein) mass proportion, due to improvements of genetic merit with increased demand for lean meat during the last two decades. Banks (2003) reported that from the late 1990s, the genetic improvement increased sheep productivity and product quality by 4% per year, thus generating a very competitive product (heavy and lean lamb carcasses; 18 to 22 kg). Sheep with high growth rates tend to have greater basal metabolism (Costa et al., 2013), and this would stimulate the activity of internal organs with greater digestive load, cardiac output and blood flow required to digest, absorb and deliver nutrients to the mammary gland, and consequently, resulting in greater oxygen consumption by the animals (Reynolds, 1996). The basal metabolic rate is mainly from the metabolism of body protein mass. ARC (1980) suggested that efficiency of ME utilization for fat deposition in ruminants was about 0.70, but the efficiency for protein deposition was only 0.45. Fasting HP per kg BW in lean pigs is significantly higher than that in fat pigs (Noblet et al., 1998). There has been increasing evidence suggesting that maintenance energy requirements per kg metabolic BW for sheep and cattle have increased with increasing animal genetic merit during the last 30 years. The maintenance energy requirement currently used to ration sheep in the UK (AFRC, 1993) was developed using calorimeter data obtained over 40 years. Therefore there is an urgent need to update the recommendation of AFRC (1993) to reflect the high basal metabolic rate of the current sheep flocks.

Effect of dietary and animal factors on maintenance energy requirement

Concentrate Supplement

A previous meta-analysis of calorimeter chamber data reported a higher maintenance energy requirement for dairy cows offered high forage diets (forage proportion > 70%) than those given high concentrate diets (forage proportion < 30%) (Dong et al., 2015a). The higher maintenance metabolic rates observed for cattle offered forage-based diets might be due to an increased energy expenditure associated with the digestive tract and other internal organs. Indeed, Steen et al. (1998) revealed that in a slaughter study with finishing lambs, sheep offered diets containing a high proportion of silage had a significantly greater mass of alimentary tract than sheep offered diets containing a high proportion of concentrates. Webster (1981), in a review of scientific literature on the sources of energy expenditure, estimated that 45% of total HP was related to the gastrointestinal organs. However, the present study showed little difference in derived NE_m values (0.363 vs. 0.371 MJ/kg^{0.75}) for sheep offered forage only diets against mixed diets of forage and concentrate (mean forage proportion = 64%). The different effects of dietary forage proportion on maintenance energy requirements between lactating dairy cows of Dong et al. (2015a) and lambs in the present might be due to that lambs in the present study had a much lower intake capacity than that (DMI = 70 vs. 148 g/kg^{0.75}) in the study of Dong et al. (2015a). Consequently, the low intake capacity in the present study might restrict the potential of the effect of dietary forage proportion on the basal metabolic rates of lambs. A further reason might be attributed to the nature of metabolism studies – the short period of feeding (24 d in the present study) might not give enough time for lambs to enlarge their internal organs to the threshold which significantly increases the basal metabolic rate.

Sheep genotype

Sheep industry in the UK is characterized by a stratified structure, which has evolved over many years to best utilise the available grassland and to match breeds or crosses to different systems (Bunger et al., 2011). The stratified system for sheep has different

breeding objectives with each strata and makes use of specialized sire to achieve heterosis and complementarities of breeds. Maternal sire sheep mainly live in hill areas or upland areas with high traits of weight (e.g., birth, weaning, post-weaning, yearling and hogget) and fleece weight (Brown et al., 2007), and terminal sire breeds are mainly bred for high lean growth which live in upland or lowland (Bunger et al., 2011). In the present study, there was no significant difference in the maintenance energy requirement between lambs bred from maternal sire against terminal sire. Similar results were also found when comparison in maintenance metabolic rates was made between sheep breeds (Salah et al., 2014; Cárdenas et al., 2018) and between dairy cow genotypes (Xue et al., 2011; Dong et al., 2015b). Therefore, AFRC (1993) does not suggest any adjustment for prediction of maintenance ME requirements between breeds of lambs or early vs. late maturing characteristics.

Physiological stage

Graham et al. (1974) showed that maintenance energy requirements of sheep decreased exponentially with increasing age and it was later adopted by SCA (1990). Similarly, AFRC (1993) gives a marginally higher fasting metabolism requirements for sheep up to 1 year old than those over 1 year old. However, the energy rationing systems of NRC (1985) and INRA (1989) give a fixed NE_m value for all sheep, assuming that there are no effects of the physiological stage on maintenance energy requirements. The present results also showed no significant difference in NE_m (0.356 vs. 0.361 MJ/kg^{0.75}) or ME_m (0.484 vs. 0.491 MJ/kg^{0.75}) between lambs and ewes. Salah et al. (2014) using data derived from 81 sheep breeds and 10,700 sheep did not find any difference in energetic efficiencies among groups of sheep at different ages (weaning to 8 months vs. 8 to 12 months vs. over 12 months).

Lamb sex

It is commonly assumed that maintenance energy requirements for female and castrated sheep are similar and are lower than that for intact males due to a high body protein

concentration in male sheep (NRC, 1985; INRA, 1989; SCA, 1990; AFRC, 1993; Luo et al., 2004). However, a range of recent studies do not support this concept. For example, Rodrigues et al. (2016), using a non-descript breed of hair lambs in a comparative slaughter experiment, suggested that NE_m values were similar between sexes of lambs (intact male vs. castrated vs. female). Deng et al. (2014) found that NE_m values for Dorper and thin-tailed Han female lambs was 5% greater than that for their male counterparts, and the NE_m for female lambs was 11% greater than that predicted by AFRC (1993) for a house female lambs, but closely to the prediction for housed intact male lambs. A similar result was also obtained in the present study that female lambs had higher NE_m (0.352 vs. 0.306 and 0.288 MJ/kg^{0.75}) or ME_m (e.g., 0.507 vs. 0.441 and 0.415 MJ/kg^{0.75}) than those for male and castrated lambs. These results might be due to that female lambs normally have lower carcass weights and greater proportions of internal organs over BW when comparing with male and castrated lambs (Crouse et al., 1981; Vargas et al., 2014). Koong et al. (1985), using the path coefficient analysis, found that internal organ masses (e.g., gastrointestinal tract, pancreas, liver and kidney) were highly and positively correlated with fasting HP of sheep. Indeed in the present study, female lambs had a higher ratio of HP over ME intake (0.73 vs. 0.69 and 0.61) when comparing with male and castrated lambs with a similar BW.

Conclusions

A range of updated maintenance energy requirement values for the current sheep flocks were developed in the present study using calorimeter data collated from a number of recent sheep studies undertaken at this Institute. The current maintenance energy requirements are much higher than those recommended by sheep energy feeding systems of AFRC (1993), which was developed using data over 40 years ago. This result indicates that using AFRC (1993) to ration current sheep flocks may underestimate their maintenance energy requirements. Therefore there is an urgent need to update the energy feeding system of AFRC (1993) to reflect the higher metabolic rates of the current sheep flocks.

Table 1. Animal (n = 131), diet (n = 73) and nutrient digestibility (n = 131) data used in the present study

Item	Mean	SD	Minimum	Maximum
Animal and dietary data				
BW, kg	42.2	9.07	29.0	69.8
DMI, kg/d	1.15	0.319	0.51	2.09
CP content, kg/kg DM	0.179	0.0365	0.091	0.227
ADF content, kg/kg DM	0.241	0.0408	0.184	0.345
NDF content, kg/kg DM	0.475	0.0505	0.382	0.544
Forage proportion, kg/kg DM	0.846	0.1840	0.448	1.000
Nutrient digestibility of diet, kg/kg				
DM	0.762	0.0561	0.658	0.875
Nitrogen	0.705	0.0733	0.426	0.851
ADF	0.805	0.0486	0.644	0.907
NDF	0.793	0.0563	0.643	0.913
OM	0.818	0.0389	0.723	0.885
Digestible OM in DM	0.762	0.0561	0.658	0.875

Table 2. Energy metabolism data (n = 131) used in the present study

Item	Mean	SD	Minimum	Maximum
Energy intake and outputs, MJ/d				
Gross energy intake	21.2	5.93	9.3	38.5
Faecal energy	4.7	1.32	1.9	8.3
Urinary energy	1.0	0.35	0.3	2.4
Methane energy	1.2	0.39	0.4	2.3
ME intake	14.3	4.56	5.6	27.7
Heat production	9.6	2.87	3.3	18.6
Energy balance	4.7	3.55	□4.6	15.9
Energy utilization efficiency, MJ/MJ				
DE/GE	0.775	0.0422	0.667	0.859
ME/GE	0.669	0.0404	0.569	0.770
ME/DE	0.863	0.0342	0.741	0.922
Heat production/ME intake	0.704	0.2310	0.288	1.619
Energy balance/ME intake	0.296	0.2310	□0.619	0.712

Table 3. The linear regression equations and the derived NE (NE_m) and ME (ME_m) requirement for maintenance^{1,2}

Equation	R ²	NE _m	ME _m	Eq. No
E _g = 0.736 _(0.0571) ME intake □ 0.358 _(0.0656)	0.765	0.358	0.486	1a
HP = 0.264 _(0.0571) ME intake □ □ 0.358 _(0.0656)	0.534	0.358		1b

¹Unit = MJ/kg^{0.75} for E_g (energy balance), ME intake, HP (heat production), ME_m and NE_m.

²Values in parentheses are SE.

Table 4. Effects of dietary and animal factors on NE (NE_m) and ME (ME_m) requirements for maintenance of sheep derived from linear regressions between energy balance (E_g) and ME intake (MEI) with a common slope within each comparison ^{1,2}

Equation			R ²	NE _m	ME _m	Eq. No
Dietary type (n = 131)						
Forage	E _g = 0.746 _(0.0618) MEI	-0.363 _(0.0668)	0.765	0.363	0.486	2a
Mixed diet		-0.371 _(0.0733)		0.371	0.498	2b
Sire type (n = 131)						
Maternal	E _g = 0.741 _(0.0581) MEI	-0.366 _(0.0680)	0.764	0.366	0.495	3a
Terminal		-0.357 _(0.0651)		0.357	0.482	3b
Physiological stage (n = 131)						
Lamb	E _g = 0.736 _(0.0574) MEI	-0.356 _(0.0794)	0.765	0.356	0.484	4a
Ewe		-0.361 _(0.0932)		0.361	0.491	4b
Lamb sex (n = 96)						
Male	E _g = 0.694 _(0.0629) MEI	-0.306 _(0.1071)	0.807	0.306	0.441	5a
Female		-0.352 _(0.1029)		0.352	0.507	5b
Castrated		-0.288 _(0.1043)		0.288	0.415	5c

¹ Values in parentheses are SE; Unit = MJ/kg^{0.75} for NE_m, ME_m, E_g and MEI

² There is no significant difference in constants between Eq. 2a and 2b, 3a and 3b or 4a and 4b, but female lamb (Eq. 5b) had a higher constant than male (Eq. 5a) and castrated (Eq. 5c) lambs (P = 0.045).

Table 5. Effects of dietary and animal factors on slopes of linear regressions between energy balance (E_g) and ME intake ($\text{MJ/kg}^{0.75}$) with a common constant within each comparison ^{1,2}

Equation			R ²	Eq. No
Dietary type (n = 131)				
Forage	E _g = 0.751 _(0.0640) ME intake	-0.366 _(0.0677)	0.766	6a
Forage+Concentrate	E _g = 0.740 _(0.0576) ME intake			6b
Sire type (n = 131)				
Maternal sire	E _g = 0.736 _(0.0573) ME intake	-0.362 _(0.0659)	0.767	7a
Terminal sire	E _g = 0.746 _(0.0620) ME intake			7b
Physiological stage (n = 131)				
Lamb	E _g = 0.710 _(0.0648) ME intake	-0.359 _(0.0692)	0.767	8a
Ewe	E _g = 0.780 _(0.0781) ME intake			8b
Lamb sex (n = 96)				
Male	E _g = 0.717 _(0.0697) ME intake	-0.329 _(0.1034)	0.807	9a
Female	E _g = 0.667 _(0.0643) ME intake			9b
Castrated	E _g = 0.735 _(0.0649) ME intake			9c

¹ Values in parentheses are SE;

² There is no significant difference in slopes between Eq. 6a and 6b, 7a and 7b or 8a and 8b, but female lamb (Eq. 5b) had a lower slope than male (Eq. 5a) and castrated (Eq. 5c) lambs ($P = 0.048$).

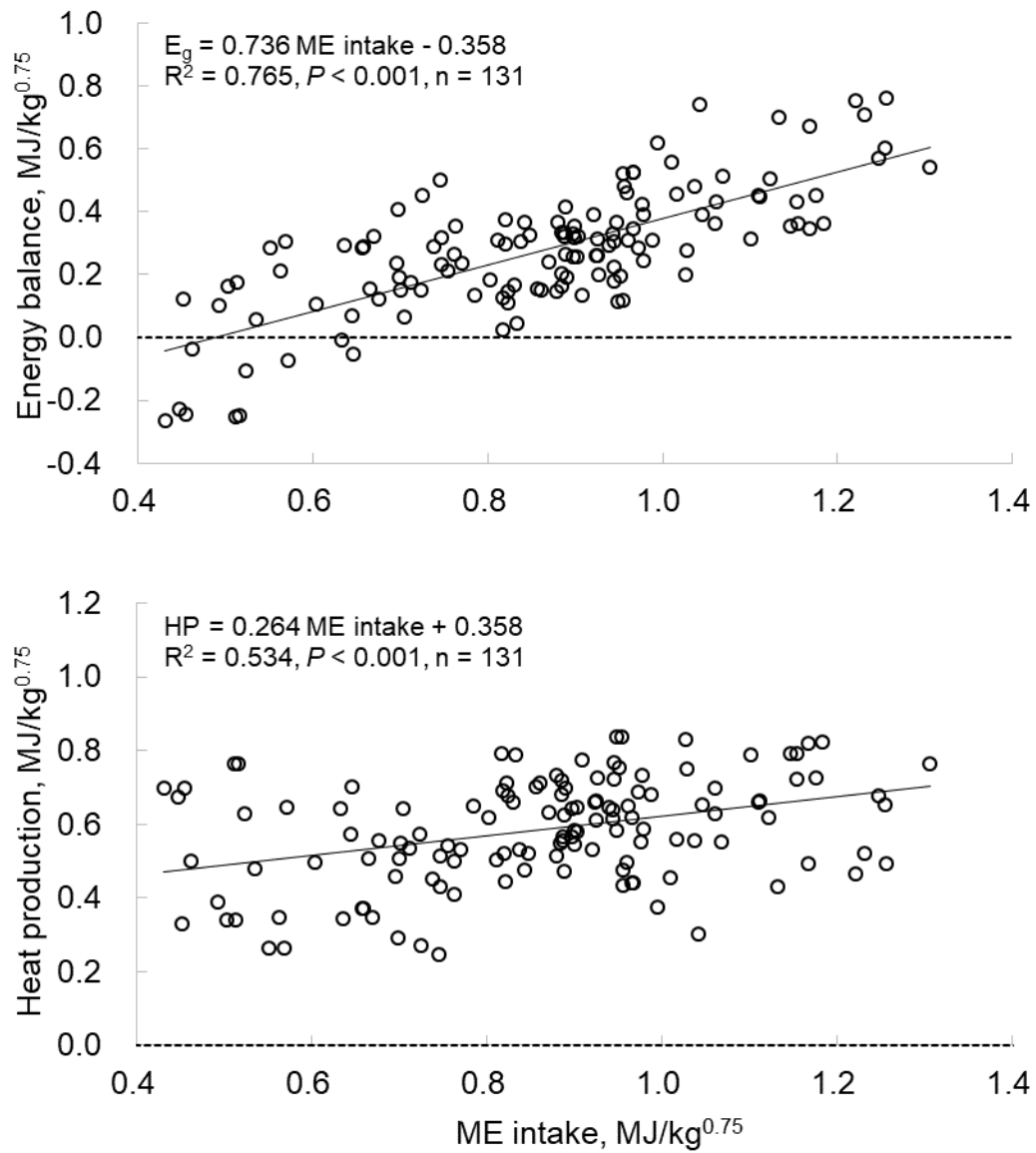


Figure 1. Linear relationships between energy balance or heat production and ME intake for sheep (n = 131).

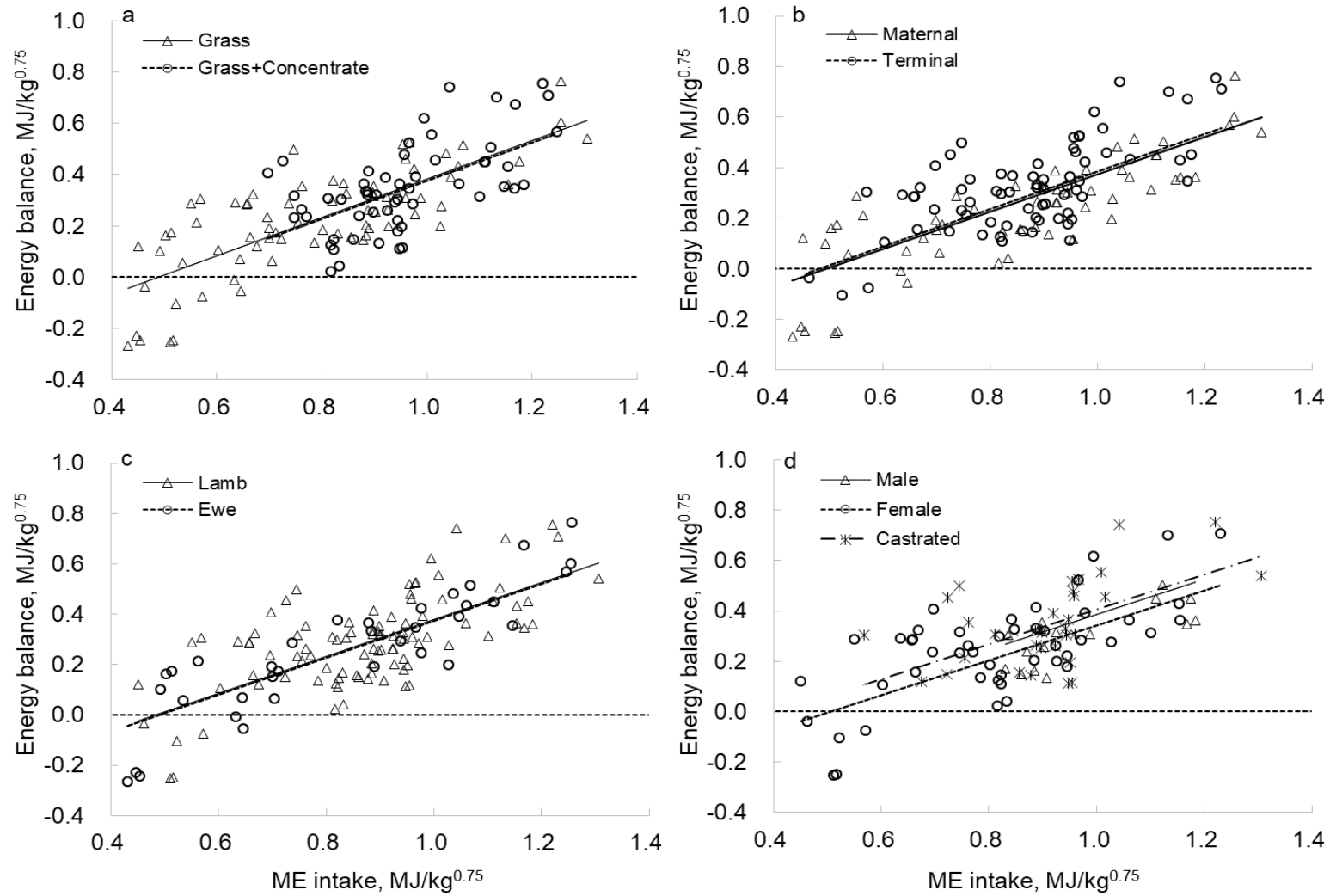


Figure 2. The effects of concentrate supplement (a), sire genotype (b), physiological stage (c) (n = 131) and lamb sex (d) (n = 96) on the linear relationship between energy balance and ME intake of sheep.

Chapter 3.

Prediction of enteric methane emissions from sheep offered fresh perennial ryegrass (*lolium perenne*) using data measured in indirect open-circuit respiration chambers

Abstract

Development of effective methane (CH₄) mitigation strategies for grazing sheep requires accurate prediction tools. The present study aimed to identify key parameters influencing enteric CH₄ emissions and develop prediction equations for enteric CH₄ emissions from sheep offered fresh grass. The data used were collected from 82 sheep offered fresh perennial ryegrass (*Lolium perenne*) as sole diets in six metabolism experiments (data from non grass-only-diets were not used). Sheep were from breeds of Highlander, Texel, Scottish Blackface and Swaledale, at age of 5 to 18 months, and weighting from 24.5 to 62.7 kg. Grass was harvested daily from 6 swards in contrasting harvest dates (May to December). Prior to the commencement of each study, the experimental sward was harvested at a residual height of 4 cm and allowed to grow for 2 to 4 weeks. The feeding trials commenced when the grass sward was suitable to zero grazing (average grass height = 15 cm), *thus, offering grass of a similar quality that grazing animals would receive under routine grazing management*. Sheep were housed in individual pens for 14 d and then moved to individual calorimeter chambers for 4 d. Feed intake, faecal and urine outputs and CH₄ emissions were measured during the final 4 d. Data were analysed using the restricted maximum likelihood procedure to develop prediction equations for CH₄ emissions. Linear and multiple prediction equations were developed using BW, DMI, GE intake (GEI) and grass chemical concentrations (DM, OM, water soluble carbohydrates (WSC), NDF, ADF, nitrogen (N), GE, DE and ME) as explanatory variables. The mean CH₄ production was 21.1 g/kg DMI or 0.062 MJ/MJ GEI. Dry matter intake and GEI were much more accurate predictors for CH₄ emissions than BW ($P < 0.001$, $r^2 = 0.86$ and 0.87 vs. 0.09). Adding grass DE and ME concentrations and grass nutrient concentrations (e.g., OM, N, GE, NDF and WSC) to the relationships between DMI or GEI and CH₄ emissions improved prediction accuracy with R^2 values increased to 0.93. Models based on farm level data, e.g., BW and grass nutrient (i.e. DM, GE, OM and N) concentrations were also developed and performed satisfactorily ($P < 0.001$, $R^2 = 0.63$). These models can contribute to improve prediction accuracy for enteric CH₄ emissions from sheep grazing on ryegrass pasture.

Introduction

The Intergovernmental Panel on Climate Change (IPCC) Tier 1 default emission factor is used in UK to estimate enteric CH₄ production for sheep with no consideration of effects of animal and dietary factors (NAEI, 2014). This may cause errors when developing national CH₄ emission inventories, because enteric CH₄ production can be influenced by diet quality, animal breed and management system (Yan et al., 2009). It is thus of highly valued to develop more accurate prediction equations specific to sheep and representative of the breeds and rearing systems employed in the UK sheep industry.

However, there is little information available in the literature of CH₄ emissions from sheep offered fresh grass with different breeds measured by respiration chambers. Pasture-based sheep production is the common management system in the cool and moist areas across the world, and the contribution of grazing animals to CH₄ emissions from the agricultural sector is distinctively important. The lack of such information may impact the development of robust national CH₄ inventories and appropriate mitigation strategies to reduce the environmental footprint of sheep production systems. Theoretically, the CH₄ conversion factor (CH₄ energy as a proportion of GE intake) currently recommended by IPCC is 6.5% and increasing feed intake may reduce CH₄ emissions per unit of feed intake. The objectives of the present study were to measure CH₄ emissions using respiration chambers and to develop prediction equations for CH₄ emissions from sheep offered fresh perennial ryegrass.

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).

Animals, experimental design, and diets

The current study collected data from six metabolism experiments (from May 2012 to June 2014) using 82 sheep including two lowland breeds (29 Highlander and 29 Texel) and two upland breeds (12 Scottish Blackface and 12 Swaledale). Animals (n = 82) were at age of 5 to 18 months, weighting from 24.5 to 62.7 kg. The six studies were designed to evaluate the effects of a range of diet (e.g., grass with or without concentrate supplementation) and animal (e.g., between breeds) factors on nutrient utilization and enteric CH₄ emission. The data used

in the preset study were collected only from sheep offered *ad libitum* fresh-cut perennial ryegrass (*Lolium perenne*) as the sole diets with no concentrate supplementation. The animals were blocked in groups with 6 sheep in each group when run through the 6 respiration chambers with one sheep per chamber in sequence in each experiment. The 82 sheep data used in the present study included 12 hill ewe lambs in Experiment (Exp) 1 (6 Scottish Blackface and 6 Swaledale, 12 month old and BW = 42.8 ± 4.26 kg), 12 hill ewe lambs in Exp 2 (6 Scottish Blackface and 6 Swaledale, 18 month old and BW = 47.8 ± 4.26 kg), 13 lowland ewe lambs in Exp 3 (7 Texel and 6 Highlander, 18 month old and BW = 51.1 ± 6.20 kg), 13 lowland growing lambs in Exp 4 (6 Texel and 7 Highlander, 6 month old and BW = 29.6 ± 2.93 kg), 24 lowland growing lambs in Exp 5 (12 Texel and 12 Highlander, 5 month old and BW = 37.9 ± 4.19 kg) (Zhao et al., 2015), and 8 lowland ewe lambs in Exp 6 (4 Texel and 4 Highlander, 14 month old and BW = 58.5 ± 4.11 kg).

Fresh grass was harvested daily in the morning from perennial ryegrass swards in the research farm at the Agri-food and Biosciences Institute (Hillsborough, Co. Down, UK; 54°27'N; 06°04'W). The Exp. 1, 2, 3, 4, 5 and 6 were undertaken in May to June 2012, September to October 2012, October to November 2012, November to December 2012, August to October 2013 and May to June 2014, respectively. Prior to the commencement of each study, the experimental sward was harvested at a residual height of 4 cm and allowed to grow for 2 to 4 weeks. The feeding trials commenced when the grass sward was suitable to zero grazing (average grass height = 15 cm), thus, offering grass of a similar quality that grazing animals would receive under routine grazing management.

Sward heights were measured throughout each experimental period using a rising plate meter (Jenquip folding plate pasture meter; Jenquip, Feilding, New Zealand), with 20 sward height measurements being taken at random in a “W” shape across the area designated for harvesting. The mean above-ground herbage masses for the cutting areas were then estimated using the following linear equation: Herbage mass (kg DM/ha) = (sward height (cm) \times 316) + 330 (Jiao et al., 2014). The required paddock size was calculated depending on the feed intake of the sheep and the herbage mass. The chemical composition of the fresh grass is shown in Table 1.

The sheep were individually housed in pens in sequence with 6 sheep for each group according to their schedule in respiration chambers and offered experimental diets for 14 d before being transferred to individual chambers for 4 d. Feed intake, faecal and urine outputs and CH₄

emissions were measured. The sheep were housed in metabolic crates which were individually placed in each chamber with one sheep per chamber. 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 fresh-cut grass and water and collect faeces and urine. The amount of fresh grass offered was adjusted based on average feed intake of the previous two days to ensure a 10% refusal.

Measurements

Quantities of feed offered and refused were recorded daily during the experiment period for each animal, and samples of fresh grass and refusals were retained daily for the measurement of DM concentration at 85°C for 24 h. Body weight was measured at the beginning of each study and before entering and after leaving the chambers. During the final 4 d when animals were housed in respiration chambers, fresh grass samples were taken daily and dried at 85°C for 24 h for determination of DM and the dried samples were bulked on a two-day basis for analysis of GE, nitrogen (N), NDF, ADF and ash. A fresh grass sample was also taken simultaneously and dried at 60°C for determination of water soluble carbohydrate (WSC) concentration.

The quantities of faeces and urine outputs were recorded daily during the 4 d in the chambers. Urine samples were acidified during collection to ensure 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 of each animal in the 4 d were thoroughly mixed separately 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 samples were used for measurement of N and GE concentration, with GE measured in 10 mL freeze-dried samples, which were contained in self-sealing polythene bags of known weight and energy concentration.

Gross energy concentrations in grass, faeces and urine were determined in an isoperibol bomb calorimeter (Parr Instruments Co., Moline, Illinois). Total N concentrations were analysed on a fresh basis for samples of faeces and urine, and on a DM basis for fresh grass samples using a Tecator Kjeldahl Auto 1030 Analyser (Foss Tecator AB, Höganäs, Sweden). Crude protein

concentration was determined as Kjeldahl N \times 6.25. 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). Grass WSC concentration was analysed using a Continuous Segmented Flow Analyser (SEAL Analytical Ltd., Southampton, UK) by the method of McDonald and Henderson (1964). Ash was measured by combustion using a muffle furnace (Vecstar Ltd., Chesterfield, UK) at 550°C for 10 h (Method 942.05, AOAC, 1990). Feeding level (FL) was calculated as ME intake divided by ME requirement for maintenance (ME_m) (AFRC, 1993).

Emissions of CH₄ were measured using sheep respiration chambers as described by Zhao et al. (2015). Six indirect open-circuit respiration chambers were used with one sheep housed per chamber. The animals 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 (Lucite International, Darwen, UK) walls fitted in aluminum frames and 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 \pm 1°C and 60 \pm 10% relative humidity, respectively. The exhaust air was removed from each chamber separately for measurement of volume, temperature, humidity and pressure. The CH₄ concentrations in the air into and out of each individual chamber were measured every 14 minutes (the interval for each chamber and the ambient air at 2 minutes) using a MGA3000 Multi-Gas Analyser (ADC Gas Analysis Ltd., Hoddesdon, Herts, UK). The analyser was calibrated weekly using oxygen free N₂ (zero gas) and a known quantity of CH₄ (span gas). This determined the absolute range (0 – 500 μ L/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, 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%.

Statistical analyses

The same structure of all experiments enabled combined analysis of data using the restricted maximum likelihood procedure to develop prediction equations for enteric CH₄ using dietary and animal factors. Linear and multiple regression techniques were used to develop prediction equations for CH₄ emissions with sex, breed and experimental period as random effects. The

prediction equations used animal (i.e., BW, DMI and GE intake) and dietary (i.e., DM, OM, WSC, NDF, ADF, N, GE, DE and ME concentrations and digestible OM in DM (DOMD)) factors as explanatory variables, where the response variables were CH₄ emissions (g/d or MJ/d) and CH₄ yield (g/kg DMI or MJ/MJ GEI). The significance of the explanatory variables fitted in the multiple linear regressions was assessed using the Wald statistic. The coefficient of determination values were estimated from pseudo coefficient of determination values using the square of the correlation between fitted values and observed values. The statistical program used in the present study was Genstat statistical package (16th edition; Lawes Agricultural Trust, Rothamsted, UK) with a probability level of $P \leq 0.05$ for significance of relationships.

Results

Grass chemical composition, intake, digestibility and CH₄ emissions

The mean, SD, minimum and maximum measured values for grass chemical composition are presented in Table 1, and for grass intake and digestibility, animal BW and CH₄ emissions are presented in Table 2. Variation for these variables was relatively great which enabled relationships to be identified between these variables and CH₄ production. For example, maximum grass NDF and ADF concentrations were approximately 1.4 times, with maximum DM and ash being doubled and N and WSC being 3-fold and 4-fold of their minimum values, respectively. Cautions should be taken for grass NDF and ADF measurements when dried above 50°C, because heat-drying of forages above 50°C can increase the yield of lignin and fibre (Van Soest, 1965). However, the GE concentrations of the grass used across all experiments were relatively consistent, ranging from 18.1 to 19.2 MJ/kg DM. In consequence, digestibility of the grass nutrient and energy concentrations ranged widely from 65% to 90%, except that the minimum N digestibility was 43%. The heaviest animal used was 38.2 kg heavier than the lightest one. Sheep average CH₄ emissions were 18.2 g/d or 1.0 MJ/d in CH₄ energy (CH₄-E) output. When expressed as per unit of feed (DM or GE) intake, the measured CH₄ production was 21.1 g/kg DMI or 0.062 MJ/MJ GEI.

The relationships between CH₄ emission rates and grass nutrient concentrations and digestibility

Correlation coefficient (r) values in linear relationships of grass nutrient concentrations, digestibility and feeding level to CH₄ emission rates (g/kg DMI and OMI or MJ/MJ GEI and DEI) are presented in Table 3. All CH₄ emission rates were negatively correlated ($P < 0.001$)

with grass DM, OM, WSC and ME concentrations, but positively correlated ($P < 0.001$) with grass GE and N concentrations. Feeding level had negative effects on CH₄ emission rates ($P < 0.001$). However there were no linear relationships between CH₄ emission rates and grass NDF and ADF concentrations and NDF digestibility, respectively ($P > 0.05$). Grass DE concentration ($P = 0.011$), DM digestibility (DMD) ($P = 0.001$) and DOMD ($P < 0.001$) had negative effects on CH₄-E output per MJ of DEI.

Single linear prediction equations for CH₄ emissions

The linear relationships between enteric CH₄ emissions and BW and feed intake variables are presented in Table 4. There were strong positive linear relationships ($P < 0.001$) between CH₄ production and DMI, OMI, digestible DM intake (DDMI) and digestible OM intake (DOMI), respectively (Eq. 1a, 1b, 1c and 1d). The relationship between CH₄ emissions and DMI is also presented in Figure 1. The DMI was the best predictor (Eq. 1a, $r^2 = 0.86$ and SE = 2.63) for CH₄ emissions (g/d) when compared with OMI, DDMI or DOMI (Eq. 1b to 1d, $r^2 = 0.83$ to 0.84 and SE = 2.84 to 3.02). Likewise, the variation in CH₄-E output (MJ/d) was best predicted by GEI rather than DEI or MEI (Eq. 2a, 2b and 2c). Although there was a positive relationship ($P = 0.006$) between CH₄ emissions and animal BW, the prediction accuracy was low with 9% of the variation in CH₄ production being accounted for by BW alone (Eq. 1e). Under the range of values at the present study, most variation in CH₄ production was predicted by DMI ($r^2 = 0.86$, Eq. 1a) and GEI ($r^2 = 0.87$, Eq. 2a) and a 1.0 kg increase in DMI was predicted to increase daily CH₄ production by 16.7 g.

Multiple linear prediction equations for CH₄ emissions using DMI or GE intake as primary predictor

As the variation in CH₄ production was best predicted by DMI and GEI, multiple linear prediction equations were developed using DMI and GEI as primary predictors, respectively, accompanied by grass nutrient (i.e., OM, N, NDF, ADF and WSC) and energy (i.e., GE, DE and ME) concentrations as supporting factors (Table 5). Positive correlations ($P < 0.001$) between CH₄ emissions and grass N, GE, DE and NDF concentrations were observed; meanwhile the correlations with grass OM, WSC and ME concentrations were negative ($P < 0.001$). Adding grass nutrient and energy concentrations as supporting predictors improved prediction accuracy with greater R^2 and lower SE than those single linear prediction equations in Table 4. The combination of DMI or GEI with grass DE and ME concentrations showed the best prediction accuracy with the same highest R^2 of 0.93 in all prediction equations.

Multiple linear prediction equations for CH₄ emissions using BW as primary predictor

Because feed intake data are not always available, especially on commercial farms, farm level data were also used to develop prediction equations for CH₄ emissions. When multiple linear predictions were developed for CH₄ emissions using BW and grass nutrient and energy concentrations (Table 6), the effect of BW and grass concentrations of DM, GE, OM, N, NDF and ADF were significant ($P < 0.001$). Positive relationships between CH₄ emissions and BW, grass DM, GE, and NDF concentrations were observed but the correlations with grass OM, N and ADF concentrations were negative. The equation developed using animal BW together with grass DM, GE, OM and N concentrations as predictors (Eq. 1t, Table 6) performed best in Table 6 with greatest R^2 and lest SE. Although the variation of CH₄ emissions was better described by intake-related variables, such as DMI and GEI (Table 4 and 5), the equations using BW and grass chemical concentrations may be important and practical at farm-level because DMI or GEI at pasture is generally not available or poorly assessed.

Single and multiple prediction equations for CH₄ yield

Prediction equations for CH₄ emissions per unit of DMI or CH₄ energy as a proportion of GEI (CH₄ yield, g/kg or MJ/MJ) were also developed by single or multiple linear regressions using FL, DMI, GEI, grass nutrient, DE and ME concentrations and DOMD (Table 7 and Figure 2). The effects of FL, DMI, GEI and grass DE, ME, NDF and N concentrations were significant ($P < 0.001$). Concentrations of DE, NDF and N had positive relationships with CH₄ yield, while FL, DMI, GEI and grass ME concentration had negative relationships with CH₄ yield. The variation in CH₄ yield was best predicted by grass DE and ME concentrations (Eq. 4g, Table 7) which agrees the results in Table 5 when they were used as supporting predictors in the relationships between DMI or GEI and CH₄ emissions per day (Eq. 1n and 2h, Table 5). The significant ($P < 0.001$) negative linear relationships between CH₄ yield and FL, DMI and GEI indicated that high intakes of fresh grass would lower CH₄ production per unit of feed intake (Eq. 3a, 3c, 4a and 4c, Table 7). Polynomial regression was also used to develop relationships between CH₄ yield (g/kg DMI or MJ/MJ GEI) and DMI, GEI or FL (Eq. 3b, 3d, 4b and 4d, Table 7, also presented in Figure 2). The result showed that polynomial regression performed better than the linear correlation which indicated the extent of CH₄ decrease was gradually slowing down rather than at a fixed rate with increasing feed intake.

Discussion

Comparison between present and published enteric CH₄ emission data

The mean CH₄ emissions from sheep offered solely fresh-cut ryegrass *ad libitum* in the current study were 21.1 g/kg DMI. This value largely agrees with other studies using respiration chambers to measure CH₄ emissions from sheep offered fresh perennial ryegrass. For example, Sun et al. (2011, 2012a, 2012b, 2015) reported CH₄ production in a range of 19.5 to 23.8 g/kg DMI, Pinares-Patiño et al. (2011) recorded a range of 22.1 to 24.9 g/kg DMI, Hammond et al. (2011, 2013, 2014) presented a range of 20.2 to 27.0 g/kg DMI and Pacheco et al. (2014) summarized a range of 18.0 to 27.0 g/kg DMI. The animals in these studies, which were designed to provide basic CH₄ emission values from grazing sheep or sheep offered fresh grass, were all offered fresh-cut perennial ryegrass, housed in individual respiration chambers and thus can provide comparable results with the present data.

In contrast, Savian et al. (2014) measured CH₄ emissions from lambs when grazing perennial ryegrass using SF₆ tracer technique, with an average CH₄ production of 19.5 g/kg DMI. This value is slightly less than the result of the current study using chambers, which mirrors possible differences between the two measurement techniques, the determination of DMI using the n-alkanes technique, and also animal behaviour indoors and outdoors. Another study carried out by Lockyer (1997) reported a CH₄ production of 13.3 g/d from sheep grazing perennial ryegrass under near natural conditions using the tunnel system. Intake was not measured in this study but was estimated to be 0.44 kg/d which averaged a CH₄ production of 30.2 g/kg DMI. This value is greater than those measured using chambers and SF₆. The difference is likely due to the falling intake with decline of leaf material as grazing progressed, which consequently resulted in an inadequate supply for maintenance requirement. Furthermore, the technical difference between chamber and tunnel system could also contribute to this disagreement. The studies cited above involve sheep of various breeds and sexes at different growing stages, producing a wide range of average daily gain, and offering ryegrass of different maturities and chemical composition, and at several feed intakes. All of these factors can influence CH₄ emissions and consequently have reasonable potential to be involved in the mitigation strategies.

The mean CH₄-E/GE obtained in the present study was 6.2% for fresh ryegrass, which is close to 6.5% of the IPCC Tier 2 value for sheep (IPCC, 2006). The IPCC Tier 2 methodology currently uses GEI, which is calculated from standard models (e.g. AFRC 1993), and a standard

CH₄ conversion factor (CH₄-E/GE = 6.5%) to calculate CH₄ emissions. However, this conversion factor which was developed using data irrespective of the influence of the nature of feed ingredients on CH₄ emissions may be used with caution for grazing animals. For example, offering fresh grass with high energy value and high digestibility (e.g. GE concentration and digestibility of 18.6 MJ/kg DM and 78.8%, respectively, in the present study) may result in less CH₄ emissions per unit of GEI than a more fibrous forage-based diet. Thus, there is still considerable room for improvement in predicting CH₄ production using factors that explain variations including effects of DMI (as it relates to BW), particle passage rate, digestion kinetics and diet chemical composition. This practice may serve for the development of Tier 3 predictions, which is recommended by IPCC to substitute Tier 2.

Prediction equations for 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*. A number of previous studies developed CH₄ prediction equations from animals offered rations based on conserved forage and concentrates (Blaxter and Clapperton, 1965; Yan et al., 2000; Ellis et al., 2007; Moraes et al., 2014). Concerns have been raised about applying these equations to grazing livestock, recommending this should be done with caution (Moraes et al., 2014). Pasture-based sheep production is the common management system in some cool and moist areas across the world capable of long grazing seasons, such as New Zealand, Ireland and UK, and the contribution of grazing animals to CH₄ emissions from the agricultural sector in these countries is distinctively important (Pacheco et al., 2014). Thus, the prediction equations using data based on fresh grass were developed using several interacting feed and animal factors in the current study.

There was a strong positive linear relationship between CH₄ production (g/d) and feed (DM, OM, DDM, DOM, GE, DE and ME) intake. Many studies have confirmed that intake level explained most of the variation in CH₄ production and it is the principle driver of methanogenesis (Johnson and Johnson, 1995; Kebreab et al., 2006; Ramin and Huhtanen, 2013). Moreover, DMI and GEI performed better than any other intake of individual chemical and energy components and their corresponding digestible and metabolizable parameters in predicting CH₄ production (Ellis et al., 2007; Yan et al., 2009; Jiao et al., 2013). Robinson et al. (2010) offered lucerne chaff to sheep at intake levels of 0.8, 1.24 and 1.6 × ME_m and found that intake level was strongly correlated with CH₄ production (L/d) ($r^2 = 0.87$). Hammond et

al. (2013) offered fresh white clover and perennial ryegrass to sheep at 0.8, 1.2, 1.6, 2.0 and $2.5 \times \text{ME}_m$ feeding levels and reported most variation in CH_4 production (g/d) was accounted for by OM intake ($r^2 = 0.87$). These results are in line with the association between DMI and CH_4 production (g/d) ($r^2 = 0.86$, Eq. 1a, Table 4) found with fresh ryegrass offered in the present study. However, the variation in CH_4 production predicted by DMI in the present study is greater than those reported in cattle (0.68 to 0.72) by Ellis et al. (2007), Jiao et al. (2013) and Yan et al. (2009).

The absolute amount of CH_4 produced increases with increased feeding level. However, increasing feed intake can reduce CH_4 production per unit of feed intake. The regression of feed intake on CH_4 yield (g/kg DMI or MJ/MJ GEI) showed a negative association, indicating that an increase of 1 kg DMI or 1 MJ GEI decreased CH_4 yield by 5.3 g/kg DMI or 0.00084 MJ/MJ GEI, respectively (Eq. 3c and 4c, Table 7). Likewise, increasing one level of intake reduced CH_4 yield by 2.4 g/kg DMI or 0.0073 MJ/MJ GEI (Eq. 3a and 4a, Table 7). The negative relationships between feeding level and CH_4 production as a proportion of DMI or GEI are mainly derived from CH_4 emission rate at maintenance level being higher than that at levels above maintenance. The dilution of maintenance is a major factor for high producing animals with a low CH_4 emissions per kg DMI (Yan et al., 2010). In other words, to produce a given amount of product, increasing animal productivity can reduce total CH_4 emissions.

Although an increase in feed intake reduced CH_4 emission yield, the extent of the reduction very much depends on the ingredients of the diet. For example, the negative relationship between feeding level and $\text{CH}_4\text{-E}/\text{GEI}$ in the present study (Eq. 4a, Table 7) indicates that the percentage of dietary GE lost as $\text{CH}_4\text{-E}$ reduced by 0.73% per level of intake, which was close to 0.78% estimated by Yan et al. (2000) in beef and dairy cattle when offered grass silage diets and 0.77% by Beauchemin and McGinn (2006) in growing beef when offered barley silage diets. However, increasing intakes of concentrate diets per level of intake reduced $\text{CH}_4\text{-E}/\text{GEI}$ by 1.6% in cattle (Johnson and Johnson, 1995) and 1.5% in sheep (Moss et al., 1995). Blaxter and Clapperton (1965) reported that the reduction in $\text{CH}_4\text{-E}/\text{GEI}$ for each multiple of ME_m intake was more for pelleted diets (2.1%) compared to forage (0.8%).

This variation is likely associated with rumen function and its regulation. A voluminous, bulky feed, such as the fresh grass in the current study would have filled the rumen to a greater degree than concentrate and finely ground (e.g. pelleted) feeds and thus reduced the intake. However,

the feed that is digested rapidly and in fine particle size such as concentrate promotes greater intake and consequently, faster passage rate than forages. Hammond et al. (2014) reported the decline in CH₄ yield as intake level increases is strongly associated with shorter rumen retention times of both solid and liquid fractions of digesta. Another explanation could be most experiments included concentrates which, in effect, lowered the NDF of the whole diet and this may have been the main driver of lowered CH₄ production when compared with forage. Thus, feeding grass with less fibre is proposed as a CH₄ mitigation strategy for pasture-based systems.

Furthermore, effects of intake level on CH₄ emissions from sheep offered fresh grass are not consistent either. Hammond et al. (2013) reported a decline of 1.3% in CH₄-E output as a proportion of GEI for every feeding level increase in sheep offered fresh-cut grass, which is greater than 0.73% that measured in the present study. This is possibly because the dataset in the study of Hammond et al. (2013) comprised of both white clover and ryegrass and the decrease in CH₄ emission yield with increasing intake levels was greater for white clover than ryegrass. Moreover, in contrast of several feed intake levels used in the study of Hammond et al. (2013), the sheep in the current study were offered grass *ad libitum* and consequently had a greater feeding level (1.9 vs. 1.5) on average. The polynomial regression (Figure 2) indicated the extent of CH₄ decrease was gradually slowing down rather than at a fixed rate with increasing feed intake. Therefore, this might explain why the CH₄ decline was less with the greater feeding level in the current study.

The equations developed using BW and combinations of grass chemical composition parameters as predictors, can be recommended in commercial practice when feed intake is not available for animals on pasture or zero-grazing diets. Positive relationship between BW and CH₄ emissions was observed, which indicates bigger animals produce more CH₄ (g/d). This relationship has been previously detected by other authors (Jiao et al., 2014; Moraes et al., 2014) and may be explained by the higher DMI in heavier animals. Moreover, the differences in gut capacity and digesta kinetics between animals of contrasting BW would influence the extent of ruminal fermentation of feeds and alter the production of volatile fatty acids; and consequently produce different amount of CH₄ (Moraes et al., 2014). The models using BW as primary predictor and grass nutrient and energy concentrations as supporting factors increased the prediction accuracy relative to the one fitted with BW as the only predictor. Therefore, including both animal BW, and grass nutrient and energy concentrations improved model goodness of fit and resulted in equations which were better supported by the observed data.

Effect of grass quality on CH₄ emissions

Grass nutrient and energy concentrations played an important role in CH₄ production as detected in the prediction equations using DMI, GEI and BW as primary predictors (Table 5 and 6). The present study resulted in a high R² (0.93) using DMI or GEI as primary predictor together with grass DE and ME concentrations in predicting CH₄ emissions (Eq. 1n and 2h, Table 5). Furthermore, CH₄ emissions were positively correlated with grass DE concentration and negatively correlated with grass ME concentration, respectively, which reflected the fact that CH₄ energy is derived as the difference between DE and a sum of ME and urine energy in ruminant animals. These relationships were also detected by Pelchen and Peters (1998) and Yan et al. (2009) in sheep and beef cattle respectively. However, the R² (0.93) value in the current study was greater than those (R² = 0.70 and 0.84, respectively) reported by Pelchen and Peters (1998) and Yan et al. (2009). This is possibly due to fresh-cut ryegrass being the only forage offered in the present study, rather than various forages or a mixture of forage and concentrates used in the studies of Pelchen and Peters (1998) and Yan et al. (2009) and consequently contributing to the development of robust prediction equations.

Grass WSC and NDF concentrations were among the main supporting predictors in a number of equations, and had negative and positive effects on CH₄ emissions, respectively. This confirmed the relationships between dietary carbohydrate types and enteric CH₄ emissions from ruminants. The fermentation of cell wall carbohydrates (e.g. NDF) results in a greater methanogenic progress in the rumen than the fermentation of non-cell wall components (e.g. WSC) (Moe and Tyrrell, 1979; Johnson and Johnson, 1995). 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 negative correlation of grass ADF concentration with CH₄ emissions in one equation (Eq. 1q, Table 6) may be explained by the confounding effect of NDF, which had a positive relationship with grass ADF, in the same equation. Likewise, negative correlations between grass ADF and CH₄ energy output in lactating cows have been reported by Ellis et al. (2007) when NDF had a positive effect in the same model. This indicated the difference between the relative proportions of NDF and ADF fractions, which is mostly hemicelluloses (Van Soest, 1967), might play an important role in producing CH₄.

Grass DOMD was found among the significant predictors in equations that predicted CH₄ per unit of DMI or GEI (Eq. 3e, 3f, 4e and 4f, Table 7). Its positive relationship to CH₄ yield may be a result of confounding effects between grass DOMD and the other predictors in the same equations, possibly grass ME concentration, rather than explaining a physiological function. The latter explanation may be supported by the relatively high positive correlation between grass ME concentration and DOMD which was $ME\ (MJ/kg\ DM) = 16.9\ DOMD\ (kg/kg\ DM)$ ($r^2 = 0.77$) using the fresh grass data in the present study. A similar formula that is commonly used to estimate the ME concentration is $ME\ (MJ/kg\ DM) = 16.0\ DOMD\ (kg/kg\ DM)$ for roughages given to ruminants (AFRC, 1993).

In contrast, negative linear relationships were observed between DMD or DOMD and CH₄-E as a proportion of DEI. Yan et al. (2010) reported that CH₄-E/GEI was negatively related to ME/GE and ME/DE in lactating dairy cows. Pacheco et al. (2014) found greater OM digestion was associated with less CH₄ per kg of digestible OM intake in sheep. Apparent digestibility or metabolizability is an indicator of diet quality. Offering diets with more digestible forage or supplementing pasture-based diets with highly fermentable grains are thus proposed as preferred CH₄ mitigation strategies for implementation into grazing systems (Sauvant and Giger-Reverdin, 2009; Cottle et al., 2011).

Conclusions

The CH₄ conversion factor (CH₄-E/GEI) for sheep offered perennial ryegrass was 6.2%. Dry matter intake and GE intake were better predictors for CH₄ emissions than BW and intake of any other individual nutrient and energy concentrations. Adding grass nutrient (i.e. WSC, NDF and OM), DE and ME concentrations to the relationships between feed intake and CH₄ emissions improved prediction accuracy. Models based on farm level data, e.g., BW and grass nutrient (i.e. DM, GE, OM and N) concentrations were also developed and performed satisfactorily. The data were derived from local fresh-cut ryegrass, sheep breeds and typical rearing system and can therefore be used to decrease the uncertainty in the development of CH₄ emission inventories and offer potential mitigation strategies to reduce the environmental footprint of pasture-based sheep production systems.

Table 1. Chemical composition of fresh-cut ryegrass (n = 82) (g/kg DM, unless otherwise stated)

Chemical composition	Mean	SD	Min	Max
DM, g/kg	155	31.3	113	237
Ash	91	18.2	57	116
GE, MJ/kg DM	18.6	0.29	18.1	19.2
N	28	7.3	13	36
NDF	499	37.9	421	594
ADF	254	17.8	209	298
WSC	156	54.0	75	292

Table 2. Animal BW, grass intake, nutrient digestibility and CH₄ emissions (n = 82)^{1,2}

	Mean	SD	Min	Max
<i>Body weight (kg) and intake (kg/d, or MJ/d)</i>				
BW	42.9	9.61	24.5	62.7
DMI	0.90	0.392	0.19	1.77
Digestible DM intake	0.73	0.327	0.16	1.44
OM intake	0.82	0.361	0.17	1.67
Digestible OM intake	0.68	0.306	0.15	1.38
GE intake	16.8	7.32	3.5	32.2
DE intake	13.3	5.97	3.0	26.2
ME intake	11.5	5.37	2.5	23.6
<i>Digestibility (kg/kg, or MJ/MJ)</i>				
DM	0.803	0.0446	0.695	0.898
N	0.729	0.0779	0.426	0.833
OM	0.823	0.0403	0.729	0.902
Digestible OM in DM	0.749	0.0424	0.654	0.845
GE	0.788	0.0472	0.681	0.883
NDF	0.800	0.0510	0.643	0.874
ADF	0.804	0.0489	0.701	0.884
<i>Methane parameters</i>				
CH ₄ , g/d	18.2	7.05	5.5	31.3
CH ₄ /DMI, g/kg	21.1	3.82	12.7	31.1
CH ₄ -E, MJ/d	1.00	0.389	0.31	1.73
CH ₄ -E/GEI, MJ/MJ	0.062	0.0112	0.039	0.092

¹ CH₄-E = methane energy; GEI = gross energy intake.

² BW = average of BW entering and BW leaving the chambers.

Table 3. Correlation coefficient (r) values for relationships of nutrient concentration, feeding level and digestibility to CH₄ emission rate (g/kg, or MJ/MJ)¹

Item	CH ₄ /DMI	CH ₄ /OMI	CH ₄ -E/GEI	CH ₄ -E/DEI
Chemical composition (g/kg DM, unless otherwise stated)				
DM, g/kg	-0.50	-0.55	-0.48	-0.51
OM	-0.41	-0.51	-0.41	-0.46
N	0.44	0.53	0.42	0.42
NDF	NS	NS	NS	NS
ADF	NS	NS	NS	NS
WSC	-0.47	-0.54	-0.44	-0.48
Energy concentration (MJ/kg DM)				
GE	0.28	0.33	0.23	0.21
DE	NS	NS	NS	-0.28
ME	-0.24	-0.30	-0.28	-0.55
Nutrient digestibility (kg/kg)				
DM digestibility	NS	NS	NS	-0.35
Digestible OM in DM	NS	NS	NS	-0.45
NDF digestibility	NS	NS	NS	NS
Feeding level	-0.53	-0.52	-0.54	-0.61

¹ CH₄-E = methane energy; DEI = digestible energy intake; Feeding level = ME intake divided by ME requirement for maintenance (AFRC, 1993); GEI = gross energy intake; OMI = organic matter intake; NS = non-significant ($P > 0.05$).

Table 4. Single linear prediction equations for CH₄ emissions (n =82)^{1,2,3,4}

Equations		SE	R ²	Eq. No.
CH ₄ , g/d	= 16.7 _(0.74) DMI + 3.1 _(0.73)	2.63	0.86	1a
	= 17.9 _(0.88) OMI + 3.5 _(0.79)	2.87	0.83	1b
	= 19.8 _(0.96) DDMI + 3.8 _(0.77)	2.84	0.84	1c
	= 20.9 _(1.10) DOMI + 4.0 _(0.82)	3.02	0.82	1d
	= 0.22 _(0.078) BW + 8.7 _(3.43)	6.76	0.09	1e
CH ₄ -E, MJ/d	= 0.050 _(0.0021) GEI + 0.17 _(0.039)	0.141	0.87	2a
	= 0.060 _(0.0029) DEI + 0.21 _(0.042)	0.156	0.84	2b
	= 0.064 _(0.0037) MEI + 0.26 _(0.047)	0.178	0.79	2c

¹ BW = average of BW entering and BW leaving the chambers.

² DDMI = digestible dry matter intake; DEI = digestible energy intake; DOMI = digestible organic matter intake; GEI = gross energy intake; MEI = metabolisable energy intake; OMI = organic matter intake.

³ The units of parameters are kg/d or MJ/d except BW (kg).

⁴ Values in subscript parentheses are SE; all relationships are significant ($P < 0.001$).

Table 5. Multiple linear prediction equations for CH₄ emissions using dry matter or gross energy intake as primary predictor (n = 82)^{1,2,3}

Equations	SE	R ²	Eq. No.
CH ₄ , = 17.3 _(0.70) DMI + 147 _(37.8) N – 1.6 _(1.37)	2.42	0.88	1f
g/d = 17.7 _(0.68) DMI – 74 _(14.6) OM + 70 _(13.1)	2.29	0.89	1g
= 17.7 _(0.66) DMI – 26.3 _(4.82) WSC + 6.3 _(0.86)	2.25	0.90	1h
= 16.4 _(0.70) DMI + 3.4 _(0.94) GE – 60 _(17.4)	2.45	0.88	1i
= 16.8 _(0.68) DMI + 3.4 _(0.89) GE – 0.79 _(0.250) ME – 51 _(16.8)	2.32	0.89	1j
= 17.6 _(0.69) DMI + 132 _(37.2) N – 0.62 _(0.257) ME + 6.6 _(3.60)	2.35	0.89	1k
= 17.7 _(0.66) DMI + 209 _(39.2) N + 26.4 _(7.36) NDF – 16.8 _(4.43)	2.26	0.90	1l
= 17.9 _(0.66) DMI – 84 _(14.5) OM + 18.7 _(6.66) NDF + 69 _(12.6)	2.20	0.90	1m
= 18.8 _(0.57) DMI + 5.0 _(0.61) DE – 4.9 _(0.54) ME – 9.9 _(3.70)	1.85	0.93	1n
CH ₄ -E, = 0.051 _(0.0021) GEI – 0.046 _(0.0145) ME + 0.74 _(0.180)	0.134	0.88	2d
MJ/d = 0.052 _(0.0020) GEI – 1.26 _(0.268) WSC + 0.33 _(0.048)	0.126	0.90	2e
= 0.052 _(0.0020) GEI – 0.039 _(0.0141) ME + 5.7 _(2.02) N + 0.48 _(0.196)	0.128	0.89	2f
= 0.053 _(0.0019) GEI – 0.031 _(0.0138) ME – 1.1 _(0.27) WSC + 0.69 _(0.165)	0.123	0.90	2g
= 0.055 _(0.0017) GEI + 0.25 _(0.034) DE – 0.26 _(0.030) ME – 0.39 _(0.204)	0.103	0.93	2h

¹ GEI = gross energy intake.

² The units of parameters are kg/kg DM or MJ/kg DM except DMI (kg/d) and GEI (MJ/d).

³ Values in subscript parentheses are SE; all relationships are significant ($P < 0.001$).

Table 6. Multiple linear prediction equations for CH₄ emissions using body weight as primary factor (n =82)^{1,2,3}

Equations	SE	R ²	Eq. No.
CH ₄ , = 0.29 _(0.075) BW + 8.8 _(2.49) GE – 157 _(47.2)	6.33	0.22	1o
g/d = 0.22 _(0.074) BW + 17.7 _(3.54) GE – 496 ₍₁₄₆₎ N – 307 _(62.7)	5.95	0.32	1p
= 0.38 _(0.076) BW + 10.3 _(2.48) GE + 126 _(31.1) NDF – 194 _(64.4) ADF – 203 _(52.5)	5.82	0.35	1q
= 0.19 _(0.068) BW + 12.6 _(2.29) GE + 0.11 _(0.022) DM – 242 _(44.2)	5.49	0.42	1r
= 0.29 _(0.064) BW + 206 _(28.2) DM + 10.7 _(2.08) GE – 240 _(52.5) OM – 7 _(64.7)	4.90	0.54	1s
= 0.34 _(0.059) BW + 151 _(28.5) DM + 24.5 _(3.70) GE – 463 _(69.9) OM – 1124 ₍₂₆₀₎ N – 23 _(58.5)	4.42	0.63	1t

¹ BW = average of BW entering and BW leaving the chambers.

² The units of parameters are kg/kg DM or MJ/kg DM except BW (kg) and DM (kg/kg).

³ Values in subscript parentheses are SE; all relationships are significant ($P < 0.001$).

Table 7. Prediction equations for CH₄ emission rate (n = 82)^{1,2,3}

Equations	SE	R ²	Eq. No.
CH ₄ /DMI, = -2.4 _(0.44) FL + 25.8 _(0.92)	3.27	0.28	3a
g/kg = 1.0 _(0.44) FL ² - 6.6 _(1.88) FL + 29.4 _(1.84)	3.18	0.32	3b
= -5.3 _(0.92) DMI + 25.8 _(0.90)	3.23	0.29	3c
= 4.4 _(2.12) DMI ² - 13.6 _(4.06) DMI + 29.0 _(1.77)	3.16	0.33	3d
= -3.1 _(0.81) ME + 70 _(20.9) DOMD + 277 _(53.4) N - 0.14 _(8.42)	3.21	0.32	3e
= -3.8 _(0.79) ME + 34 _(10.4) NDF + 375 _(58.3) N + 94 _(20.9) DOMD - 28 _(11.6)	3.02	0.41	3f
= 7.9 _(0.74) DE - 7.3 _(0.65) ME - 2.7 _(4.63)	2.39	0.62	3g
CH ₄ -E/GEI, = -0.0073 _(0.00127) FL + 0.08 _(0.003)	0.0094	0.29	4a
MJ/MJ = 0.0031 _(0.00126) FL ² - 0.02 _(0.005) FL + 0.087 _(0.0053)	0.0092	0.34	4b
= -0.00084 _(0.000143) GEI + 0.08 _(0.003)	0.0094	0.30	4c
= 0.000037 _(0.0000177) GEI ² - 0.0021 _(0.00064) GEI + 0.086 _(0.0052)	0.0092	0.34	4d
= -0.011 _(0.0023) ME + 0.25 _(0.059) DOMD + 0.80 _(0.152) N - 0.009 _(0.0240)	0.0091	0.36	4e
= -0.013 _(0.0023) ME + 0.32 _(0.059) DOMD + 0.10 _(0.029) NDF + 1.1 _(0.17) N - 0.09 _(0.033)	0.0086	0.44	4f
= 0.022 _(0.0022) DE - 0.021 _(0.0019) ME - 0.0001 _(0.0140)	0.0072	0.59	4g

¹ CH₄-E = methane energy; DOMD = digestible organic matter in dry matter; FL = feeding level = ME intake divided by ME requirement for maintenance (AFRC, 1993); GEI = gross energy intake.

² The units of parameters are kg/kg DM or MJ/kg DM except DMI (kg/d) and GEI (MJ/d).

³ Values in subscript parentheses are SE; all relationships are significant ($P < 0.001$).

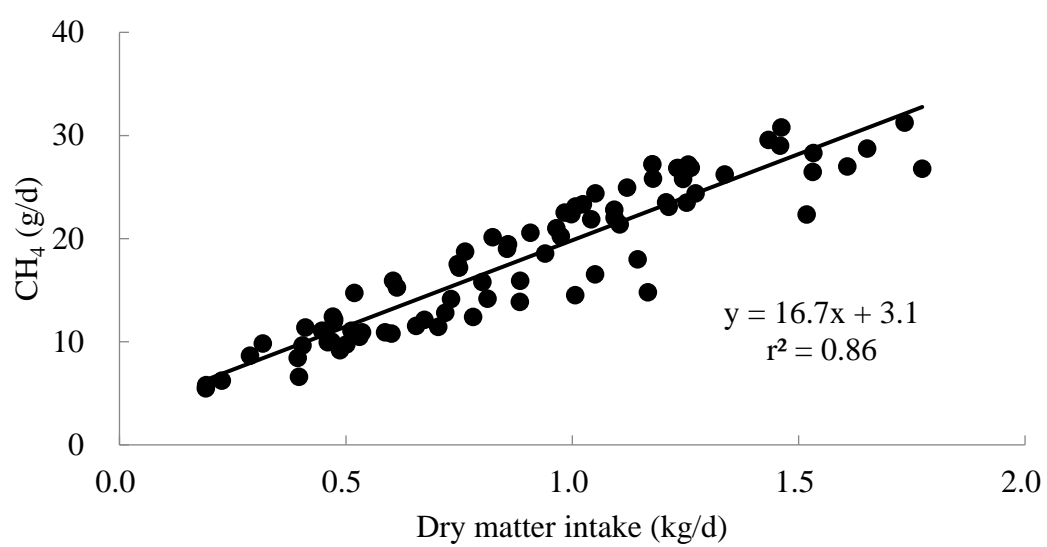


Figure 1. The relationship between CH₄ emissions and DMI (n=82)

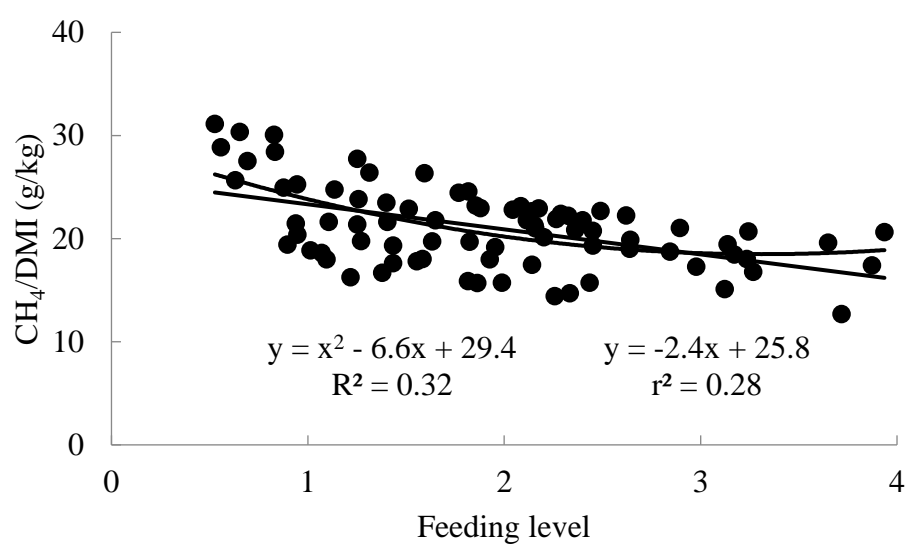


Figure 2. The relationship between CH₄ emissions per kg DMI and feeding level (n=82)

Chapter 4.

Nitrogen utilization efficiency and prediction of nitrogen excretion in sheep offered fresh perennial ryegrass (*lolium perenne*)

Abstract

Nitrogen (N) excretion from sheep production systems is an important source of nitrate, ammonia and nitrous oxide responsible for groundwater pollution and global warming. The present study aimed to identify key parameters influencing N utilization efficiency and develop prediction equations for manure N, faeces N and urine N outputs in sheep. Data used were collected from 82 sheep offered fresh perennial ryegrass (*Lolium perenne*) as the sole diet in six metabolism experiments (data from non-grass only diets were not used). Sheep were from breeds of Highlander, Texel, Scottish Blackface and Swaledale, at age of 5 to 18 months, and weighting from 24.5 to 62.7 kg. Herbage was harvested daily from 6 swards of contrasting harvest dates (May to December), offering wide variation in feed value to cover the range that would be offered in most practical farm situations. Before the commencement of each study, the experimental sward was harvested at a residual height of 4 cm and allowed to grow for 2 to 4 weeks to target an average pregrazing sward height in a range of 8 – 15 cm depending on time of year. The feeding trials commenced when the herbage sward was suitable to zero grazing, thus, offering herbage of a similar quality that grazing animals would receive under routine grazing management. Sheep were housed in individual pens for 14 d and then transferred to individual metabolism crates for 4 d with feed intake and faeces and urine outputs measured. Data were analysed using the linear mixed model procedure to develop prediction equations for faeces N, urine N and manure N using N intake, herbage chemical composition and digestibility with effects of sex, breed and experimental periods removed. Nitrogen intake was the best single predictor for N output in faeces, urine and manure, and the r^2 value for prediction of manure N output was greater than those for faeces N and urine N (0.86 vs. 0.70 and 0.77, respectively, $P < 0.001$). Animal BW and herbage DM, ether extract, NDF, ADF, water soluble carbohydrate and DE concentrations and N digestibility, instead of N intake, were also used to predict N outputs because N intake may not be available in commercial practice. The prediction equations for N utilization efficiency indicated that increasing feeding level and ME concentration and reducing N concentration could improve N utilization efficiency and shift N excretion into faeces rather than urine ($P < 0.001$). The equations

developed in the current study therefore provided an approach for sheep producers to quantify N excretion against production and consequently to develop their own mitigation strategies to reduce the environment impact from sheep production systems.

Introduction

Livestock urine and faeces are important components of the Nitrogen (N) cycle in pastures, where the microbial processes in the soil produce nitrate, ammonia and nitrous oxide which are responsible for groundwater pollution and global warming, respectively. The European Union introduced the Nitrates Directives (European Commission, 1991) that the amount of livestock manure applied to the land each year shall not exceed the amount of manure containing 170 kg N per hectare, although, producers can apply for a derogation to increase stocking rate to the equivalent of 250 kg organic N per hectare annually. Estimates of N excretion by sheep are required for developing nutrient management plans that minimize the loss of fertilizer N on pasture. Accurate information regarding N excretion could also assist sheep farmers to identify management practices that reduce the impact of sheep feeding operations on the environment. A number of previous studies developed N excretion prediction equations from cattle (Yan *et al.*, 2007; Huhtanen *et al.*, 2008; Waldrip *et al.*, 2013; Dong *et al.*, 2014) and sheep (Patra, 2010) offered rations based on conserved forage and concentrates. Stergiadis *et al.* (2015) reported several equations predicting N output of nonpregnant dry cows offered solely fresh cut herbage at maintenance levels. However, these results may not be suitable for the grazing sheep production systems due to the different diets, animal breeds and rearing systems. Pasture-based sheep production is the common management system in the cool and moist areas across the world capable of long grazing seasons. Well-managed temperate pasture often provides excess N relative to dietary energy supply (Litherland and Lambert, 2007). This leads to a low efficiency of incorporating feed N into product N (e.g., milk or meat N), and large outputs of surplus N (mainly urine) to the environment (Cheng *et al.*, 2013). However, there is little relative information available in predicting N utilization efficiency and excretion in grazing sheep. The lack of such information can impact the development of appropriate mitigation strategies to reduce the environmental footprint for sheep production. Therefore, the objectives of the present study were to investigate key parameters influencing N utilization efficiency and develop prediction equations for N excretion in sheep offered fresh perennial ryegrass.

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).

Animals, experimental design, and diets

The current study collected data from six metabolism experiments (from May 2012 to June 2014) using 82 sheep including two lowland breeds (29 Highlander and 29 Texel) and two upland breeds (12 Scottish Blackface and 12 Swaledale). Animals ($n = 82$) were at age of 5 to 18 months, weighting from 24.5 to 62.7 kg. The six studies were designed to evaluate the effects of a range of diet (e.g., herbage with or without concentrate supplementation) and animal (e.g., between breeds) factors on nutrient utilization. The data used in the preset study were collected only from sheep offered *ad libitum* fresh-cut perennial ryegrass (*Lolium perenne*) as the sole diets with no concentrate supplementation. The animals were blocked in groups with 6 sheep in each group when run through the 6 metabolism crates with one sheep per crate in sequence in each experiment. The 82 sheep data used in the present study included 12 hill ewe lambs in Experiment (Exp) 1 (6 Scottish Blackface and 6 Swaledale, 12 month old and $BW = 42.8 \pm 4.26$ kg), 12 hill ewe lambs in Exp 2 (6 Scottish Blackface and 6 Swaledale, 18 month old and $BW = 47.8 \pm 4.26$ kg), 13 lowland ewe lambs in Exp 3 (7 Texel and 6 Highlander, 18 month old and $BW = 51.1 \pm 6.20$ kg), 13 lowland growing lambs in Exp 4 (6 Texel and 7 Highlander, 6 month old and $BW = 29.6 \pm 2.93$ kg), 24 lowland growing lambs in Exp 5 (12 Texel and 12 Highlander, 5 month old and $BW = 37.9 \pm 4.19$ kg), and 8 lowland ewe lambs in Exp 6 (4 Texel and 4 Highlander, 14 month old and $BW = 58.5 \pm 4.11$ kg).

Fresh herbage was harvested daily in the morning from 6 perennial ryegrass swards in the research farm at the Agri-food and Biosciences Institute (Hillsborough, Co. Down, UK; 54°27'N; 06°04'W). The experiments 1, 2, 3, 4, 5 and 6 were undertaken in May to June 2012, September to October 2012, October to November 2012, November to December 2012, August to October 2013 and May to June 2014, respectively. The herbage harvested consisted of two first regrowth, two second regrowth and two winter swards. A broad range of fresh-cut herbage quality was offered to sheep, as a result of harvesting 6 perennial ryegrass swards of contrasting

harvest seasons and maturity stages, which are highly influential to herbage nutritive value. The wide variation in feed value was designed to cover the range that would be offered in most practical farm situations. Before the commencement of each study, the experimental sward was harvested at a residual height of 4 cm and allowed to grow for 2 to 4 weeks to target an average pregrazing sward height in a range of 8 – 15 cm depending on time of year. The feeding trials commenced when the herbage sward was suitable to zero grazing, thus, offering herbage of a similar quality that grazing animals would receive under routine grazing management.

Sward heights were measured throughout each experimental period using a rising plate meter (Jenquip folding plate pasture meter; Jenquip, Feilding, New Zealand), with 20 sward height measurements being taken at random in a “W” shape across the area designated for harvesting. The mean above-ground herbage masses for the cutting areas were then estimated using the following linear equation: Herbage mass (kg DM/ha) = (sward height (cm) × 316) + 330 (Jiao et al., 2014). The required paddock size was calculated depending on the feed intake of the sheep and the herbage mass. The chemical composition of the fresh herbage is shown in Table 1.

The sheep were individually housed in pens in sequence with 6 sheep for each group according to their schedule in metabolism crates and offered experimental diets for 14 days before being transferred to individual crates for 4 days with feed intake, faeces and urine outputs and methane (CH₄) emissions measured. Each crate contained a feed bin, drinking water container and separate trays to collect faeces and urine. Methane emissions were measured using sheep respiration chambers. The metabolism crates were housed in respiration chambers with one crate per chamber. The chambers were opened once daily at 0900 h to deliver fresh-cut herbage and water and collect faeces and urine. The amount of fresh herbage offered was adjusted based on average feed intake of the previous two days to ensure a 10% refusal.

Measurements

Quantities of feed offered and refused were recorded daily during the experiment period for each animal, and samples of fresh herbage and refusals were retained daily for the determination of DM concentration at 85°C for 24 h. Body weight was measured at the beginning of each study and before entering and after leaving the crates. During the final 4 d

when animals were housed in metabolism crates, fresh herbage samples were taken daily and dried at 85°C for 24 h for determination of DM and the dried samples were bulked on a two-day basis for analysis of GE, N, NDF, ADF, ash and ether extract (EE). A fresh herbage sample was also taken simultaneously and dried at 60°C for determination of water soluble carbohydrate (WSC) concentration.

The quantities of faeces and urine outputs were recorded daily during the 4 d in the metabolism crates. Urine samples were acidified during collection to ensure 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 of each animal in the 4 d were thoroughly mixed separately 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, ash and EE. Urine samples were used for measurement of N and GE concentration, with GE measured in 10 mL freeze-dried samples, which were contained in self-sealing polythene bags of known weight and energy concentration.

Gross energy concentrations in herbage, faeces and urine were determined in an isoperibol bomb calorimeter (Parr Instruments Co., Moline, Illinois). Total N concentrations were analysed on a fresh basis for samples of faeces and urine, and on a DM basis for fresh herbage samples using a Tecator Kjeldahl Auto 1030 Analyser (Foss Tecator AB, Höganäs, Sweden). The concentrations of NDF and ADF were determined using the Tecator Fibertec System (Foss Tecator AB, Höganäs, Sweden) following procedures of Robertson and Van Soest (1981). Herbage WSC concentration was analysed using a Continuous Segmented Flow Analyzer (SEAL Analytical Ltd., Southampton, UK) by the method of McDonald and Henderson (1964). Ash was measured by combustion using a muffle furnace (Vecstar Ltd., Chesterfield, UK) at 550°C for 10 h (Method 942.05, AOAC, 1990). Ether extract concentration was measured using Foss Soxtec 2043 Fat Extraction System (Foss Tecator AB, Höganäs, Sweden). Feeding level (FL) was calculated as ME intake divided by ME requirement for maintenance (ME_m), while ME_m = Net energy requirement for maintenance (NE_m)/ the efficiencies of ME use for maintenance (k_m). The equation used to calculate NE_m (MJ/d) = C₁C₂ (BW/1.08)^{0.75} + C₃ BW,

where $C_1 = 1.15$ for entire ram lambs and 1.0 for females and castrates, $C_2 = 0.25$ for up to 1 year of age and 0.23 for over 1 year old, $C_3 = 0.0067$ for housed fattening lambs. The equation used to calculate $k_m = 0.35 \text{ ME/GE} + 0.503$ (AFRC, 1993).

Emissions of CH_4 were measured using sheep respiration chambers as described by Zhao et al. (2015). Manure N (MN) output refers to the sum of faeces N (FN) output and urine N (UN) output in the current study, because total daily faeces and urine collection was performed in separate containers. Digestible OM in total DM (DOMD) was calculated using OM intake multiplied by OM digestibility and then divided by total DMI. Herbage ME concentration was calculated as the difference between measured GE intake and a sum of measured energy outputs in faeces, urine and CH_4 emissions. Data of grass nutrient intake and CH_4 emissions were reported in another paper (Zhao et al., 2016).

Statistical analyses

The same structure of all experiments enabled combined analysis of data using the linear mixed model (LMM) procedure to develop prediction equations for N excretion and utilization efficiency using dietary and animal factors. Linear and multiple regression techniques were used to develop prediction equations with sex, breed and experimental period as random effects. The prediction equations used N intake (NI), together with herbage chemical composition (i.e. DM, OM, WSC, NDF, ADF, N, EE, GE, DE and ME concentrations) and digestibility (i.e. DM digestibility (DMD), DOMD, N digestibility (ND), NDF digestibility (NDFD), ADF digestibility (ADFD) and GE digestibility (GED)) as explanatory variables, where the response variables were FN, UN, MN, FN/NI, UN/NI and UN/MN. The first step was undertaken in order to produce easy-to-use models with only one predictor, such as NI and N concentration. The second step offered the opportunity to improve prediction accuracy but in expense of model complexity, because models would also include energy and nutrient concentrations of fresh herbage. In the third step, models were developed using digestibility data, whilst in the last step, models were developed with both herbage chemical composition and digestibility. The significance of the explanatory variables fitted in the multiple linear regressions was assessed using the Wald statistic. Backward elimination was used to remove non-significant ($P > 0.05$) variables. This process also checked for potential collinearity problems by looking at the variance inflation factor (VIF) for any variable in the model. Any

explanatory variable with a VIF greater than 10 was removed from the final multivariable model in each case. The coefficient of determination (R^2) values were estimated from pseudo coefficient of determination values using the square of the correlation between fitted values and observed values. An internal cross validation was carried out to validate prediction equations developed in the current study. For each model we used leave one out cross validation using the model selected to generate fitted values to calculate the cross-validated pseudo R^2 . This is done by omitting each data point in turn and calculating the fitted value for this point. The statistical program used in the present study was Genstat statistical package (16th edition; Lawes Agricultural Trust, Rothamsted, UK) with a probability level of $P < 0.05$ for significance of relationships.

Results

Herbage chemical composition and N utilization

The mean, SD, minimum and maximum measured values for animal BW, herbage chemical composition, digestibility, DMI, N intake, outputs and utilization efficiency are presented in Table 1. There was a wide variation in these variables which enabled relationships to be identified between explanatory variables and the response variables. For example, maximum herbage NDF and ADF concentrations were approximately 1.4 times, with maximum DM and ash being doubled and EE, N and WSC being 2.5, 3-fold and 4-fold of their minimum values, respectively. The greatest values for NI, FN, UN and MN were in a range of 5 to 10 times greater than the lowest values. However, the GE concentrations of the herbage used across all experiments were relatively consistent, ranging from 18.1 to 19.2 MJ/kg DM. The heaviest animal used was 38.2 kg heavier than the lightest one. Average FN/NI, UN/NI, MN/NI and UN/FN were 0.27, 0.54, 0.81 and 2.2, respectively, which were used as N utilization efficiency indicators.

Relationships of herbage chemical composition, digestibility and feeding level to N utilization efficiency

Correlation coefficient (r) values in linear relationships of herbage nutrient concentrations, digestibility and feeding level to N utilization efficiency are presented in Table 2. Herbage DM, OM, NDF and WSC concentrations were all positively correlated ($P < 0.001$) with FN/NI but

negatively correlated ($P < 0.001$) with UN/NI and UN/MN. In contrast, herbage N, EE, GE and DE concentrations were all negatively correlated ($P < 0.001$) with FN/NI but positively correlated ($P < 0.001$) with UN/MN. Nitrogen digestibility had positive relationship with UN/MN and negative relationship with MN/NI ($P < 0.001$), respectively, however had no effect on UN/NI. Feeding level had negative relationships with FN/NI, UN/NI, MN/NI and UN/MN, respectively ($P < 0.001$).

Prediction equations for N excretion using N intake, herbage chemical composition and digestibility

There were strong positive linear relationships ($P < 0.001$) between NI and FN, UN and MN, respectively (Eq. 1a, 2a and 3a, Table 3). The relationships between N excretion and NI are also presented in Figure 1. Nitrogen intake was the best predictor for FN, UN and MN outputs when compared with any other nutrient intake. Under the range of values at the present study, a 1.0 g increase in NI was predicted to increase FN, UN and MN outputs by 0.12 g, 0.45 g and 0.59 g, respectively. Multiple linear prediction equations for FN, UN and MN were also developed using NI as primary predictor, respectively, accompanied by herbage chemical composition (i.e. DM, OM, WSC, NDF, ADF, N, EE, GE, DE and ME concentrations) and digestibility (i.e. DMD, DOMD, ND, NDFD, ADFD and GED) as supporting factors (Table 3). Positive correlation ($P < 0.001$) between herbage carbohydrate (i.e. WSC, NDF and ADF) concentrations and FN was observed (Eq. 1b and 1d, Table 3); meanwhile their correlations with UN and MN were all negative ($P < 0.001$) (Eq. 2b, 2d and 3d, Table 3). Herbage energy (i.e. GE, DE and ME) concentrations had negative ($P < 0.001$) effects on all of FN, UN and MN outputs (Eq. 1b, 1d, 2b, 2d, 3b and 3d, Table 3). Likewise, herbage DOMD played a negative ($P < 0.001$) role in N excretion (Eq. 2c and 3c, Table 3) except that FN output was not affected ($P > 0.05$). However, ND was found negatively ($P < 0.001$) correlated with FN and MN but positively ($P < 0.001$) correlated with UN (Eq. 1c, 1d, 2c and 3d, Table 3). Adding herbage chemical concentrations and digestibility as supporting predictors improved prediction accuracy with greater R^2 and less SE than those fitted with NI as the only predictor for FN, UN and MN outputs. The combination of NI with herbage EE, WSC, ME concentrations and ADFD and ND showed the best prediction accuracy in FN ($R^2 = 0.96$, Eq. 1d, Table 3). Using DOMD, NDFD and ND as supporting factors to NI resulted in the greatest R^2 (0.82) in

prediction of UN (Eq. 2c, Table 3). Similarly, the variation ($R^2 = 0.90$) in MN output was best predicted by NI and DOMD (Eq. 3c, Table 3).

Prediction equations for N excretion using BW, herbage chemical composition and digestibility

Because feed intake data are not always available, especially on commercial farms, farm level data were also used to develop prediction equations for N excretion. Herbage DM, WSC, NDF and ADF concentrations had negative ($P < 0.05$) relationships with UN (Eq. 2e, Table 4) and MN (Eq. 3e and 3f, Table 4). In contrast, herbage EE concentration had positive ($P < 0.05$) relationships with N excretion (FN, UN and MN) (Eq. 1e, 1f, 2e, 3e and 3f, Table 4). Negative relationships ($P < 0.05$) were observed between ND and FN (Eq. 1f, Table 4) and MN (Eq. 3f, Table 4), respectively, while the correlation between ND and UN (Eq. 2f, Table 4) was positive ($P < 0.05$). The equation developed using animal BW together with herbage EE and ND as predictors (Eq. 1f, Table 4) resulted in the best accuracy in predicting FN. The equations used herbage DM, EE, NDF, and WSC concentrations as predictors for UN (Eq. 2e, Table 4) and with the addition of ND and ADF (instead of NDF) for MN (Eq. 3f, Table 4) performed best with greatest R^2 and lest SE, respectively. Although the variation of N excretion was better described by intake-related variables, such as NI (Table 3), the equations using BW, herbage chemical concentrations and digestibility may be important and practical at farm-level because NI at pasture is generally not available or poorly assessed.

Prediction equations for N utilization efficiency using herbage chemical composition, digestibility and feeding level

Prediction equations for FN/NI, UN/NI, MN/NI and UN/FN were also developed using herbage nutrient and energy concentrations, digestibility and FL (Table 5). Herbage N concentration and ADFD had negative ($P < 0.05$) relationships with FN/NI (Eq. 4b, 4c and 4e, Table 5), however positive ($P < 0.05$) relationships with UN/NI (Eq. 5c and 5e, Table 5), MN/NI (Eq. 6c and 6e, Table 5) and UN/FN (Eq. 7c, 7d and 7e, Table 5) respectively. Similarly, DOMD had negative ($P < 0.05$) effect on FN/NI (Eq. 4c, Table 5) but positive ($P < 0.05$) effects on UN/FN (Eq. 7d, Table 5). Nitrogen excretion rate (FN/NI, UN/NI, MN/NI and UN/FN) was negatively ($P < 0.05$) associated with herbage GE and ME concentrations (Eq.

4e, 5e, 6e and 7f, Table 5). The variation in UN/Ni, MN/Ni and UN/FN were all best explained by herbage DM, EE, ASH, GE, ME, ADFD and ND (without ND in UN/Ni) when used herbage chemical composition and digestibility as predictors (Eq. 5e, 6e and 7e, Table 5), while the variation in FN/Ni was best predicted by herbage ADF, ASH, GE and DE (Eq. 4d, Table 5). The significant ($P < 0.001$) negative linear relationships between FL and FN/Ni, UN/Ni, MN/Ni and UN/FN (Eq. 4a, 5a, 6a and 7a, Table 5) indicated that high intake of fresh herbage would not only lower faeces N and urine N excretion per unit of N intake, but the N reduction extent in urine was greater than that in faeces. Single quadratic regressions between the same dependent and independent variables were also developed and improved models goodness of fit and resulted in equations which are better supported by the observed data in predicting UN/Ni, MN/Ni and UN/FN (Eq. 5b, 6b and 7b, Table 5). This might indicate the extent of urine N and manure N decrease and the shift of N excretion from urine to feces were gradually slowing down rather than at a fixed rate with increasing feed intake.

Discussion

Comparison between present and published N excretion data

Nitrogen excretion in faeces and urine represents a considerable loss from ruminant production systems (Castillo et al., 2000; Waldrip et al., 2013). Manure N per N intake was calculated from N intake and output in faeces and urine ranging from 84% to 93% in nonlactating cows and ranging from 69% to 73% in lactating cows, respectively (Wilkerson *et al.*, 1997). Jiao et al., (2014) reported manure N outputs in a range of 57% to 76% of N intake in young Holstein cattle. Mikolayunas et al. (2011) found manure N of lactating dairy ewes ranging from 73% to 87% of N intake when offered different percentages of orchardgrass:alfalfa. Using a data set summarizing 44 published studies, Patra (2010) calculated manure N as being approximately 86 % of total N intake in sheep offered diets containing foliages. This value is higher than 81% that obtained in the present study and out of the range from 75% to 85% reported by Molle et al. (2009) in lactating sheep offered different grass-legume mixtures. Furthermore, the average ratio of UN/FN reported by Patra (2010) was 0.54 which is much lower than the results of Molle et al. (2009) (1.9), Seip et al. (2011) (1.5), Cheng et al. (2013) (1.5) and the present study (2.2) in sheep. This is possibly due to inclusion of foliages in the diets in Patra's study shifted N excretion from urine to faeces (Patra, 2010). Data from these studies (Wilkerson et al., 1997; Cabiddu et al., 2009; Patra, 2010; Mikolayunas et al., 2011; Jiao et al., 2014) suggested that a

single data set for manure N excretion could not be used to estimate the N loss in manure in all situations as the results varying from 57% to 93% in cattle and from 73% to 87% in sheep. Similarly, the N partitioning between faeces and urine can also differ extensively. This difference is likely due to the animals used were of various production levels (e.g., lactation vs. non-lactation) (Wilkerson et al., 1997) and from different breeds (e.g., beef cattle vs. dairy cows) (Yan et al., 2006, 2007) and the diets offered differed in ingredient and chemical composition (e.g., conserved forage and concentrate diets vs. fresh herbage) (Stergiadis et al., 2015). All of these factors are likely to attribute to reasonable difference in N utilization and partitioning between faeces and urine.

Prediction equations for N excretion

There are positive relationships between NI and FN and UN excretion, respectively. N intake has been suggested as a better predictor for FN and UN excretion than other animal and dietary factors such as DMI, dietary CP concentration and BW in beef cattle and dairy cows (Waldrip et al., 2013; Dong et al., 2014; Jiao et al., 2014). With an increase in NI by 1 g, FN and UN are increased by 0.20 g and 0.68 g in lactating dairy cows (Huhtanen et al., 2008), 0.15 g and 0.56 g (Waldrip et al., 2013) or 0.20 g and 0.51 g in beef cattle (Dong et al., 2014) and 0.29 g and 0.48 g in young Holstein cattle (Jiao et al., 2014), respectively. In the current study, the N intake lost in faeces and urine was increased by 0.12 g and 0.45 g, respectively, with an increase in NI by 1 g. Using NI as a single predictor for MN output produced a high r^2 (0.86), which is comparable to those in sheep (0.86) (Patra, 2010), young Holstein cattle (0.86) (Jiao et al., 2014), beef cattle (0.90) (Yan et al., 2007) and lactating dairy cows (0.90) (Yan et al., 2006). Furthermore, using NI as the single independent variable resulted in a better-fit equation in estimating MN excretion compared with predicting FN and UN outputs ($r^2 = 0.86$ vs. 0.70 and 0.77, Eq. 1a, 2a and 3a, Table 3 and Figure 1), respectively, in the current study. This is also confirmed by the previous studies in beef cattle, non-lactating and lactating dairy cows (Huhtanen et al., 2008; Waldrip et al., 2013; Jiao et al., 2014).

Adding herbage chemical composition and digestibility parameters in the regression using NI as primary predictor in a backward elimination approach improved the R^2 in predicting FN, UN and MN excretion, respectively. The relationships between NI and N excretion were significantly influenced by dietary carbohydrate (e.g. WSC, NDF and ADF) and energy (e.g.

GE and ME) concentrations (Table 3). Some previous studies have demonstrated that N excretion is predicted more precisely using models with DMI, BW, dietary N, NDF concentrations and NI as independent variables rather than NI alone in beef cattle (Yan et al., 2007) and dairy cows (Wilkerson et al., 1997; Huhtanen et al., 2008; Stergiadis et al., 2015). Although MN, FN, and UN can be satisfactorily predicted by NI and herbage chemical composition in the current study, equations including DOMD or ND as additive predictors further improved R^2 and reduced SE, with the impact being greater in case of FN (Eq. 1c and 1d, Table 3) than in case of UN (Eq. 2c, Table 3). The contribution of DOMD and ND in the explained variation of N excretion is possibly because they represent fermentable OM and degradable N available to rumen microbes for microbial protein synthesis more accurately than herbage nutrient concentrations.

The equations developed using BW, herbage chemical composition and digestibility parameters as predictors, can be recommended in commercial practice when NI is not available for animals on pasture or zero-grazing diets. Nitrogen excretion (FN, UN and MN) could not be predicted by BW alone. However, when herbage chemical composition (DM, EE, DE, WSC, NDF and ADF) and digestibility (DOMD, ND and NDFD) were all considered as explanatory variables, the prediction accuracy of equations were improved (Table 4). Body weight alone is a poor predictor for N excretion in manure has been reported for dry and lactating dairy cows (Nennich et al., 2005; Yan et al., 2006; Stergiadis et al., 2015). A combination of BW and dietary chemical composition (i.e. N and NDF) parameters has been previously described as an accurate method to predict manure N in growing and replacement cattle (Wilkerson et al., 1997), beef cattle (Yan et al., 2007) and dairy cows (Jiao et al., 2014).

Mitigation strategies to reduce N excretion

The prediction equations obtained in the present study indicated that manipulating dietary N concentration could be an effective strategy to reduce N excretion. There were positive relationships between N excretion rate (e.g. UN/NI and MN/NI) and dietary N concentration which has also been reported in dairy cows and beef cattle (Hristov et al., 2004; Yan et al., 2006; Waldrup et al., 2013). Molle et al. (2009) found that N utilization efficiency was negatively correlated with NI and dietary N concentration in early-mid lactation sheep. Lactating goats have been reported to reduce urea N as a percentage of total UN 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 CP in dairy ewes (Mikolayunas et al., 2011). These findings together with the results in the current study indicated that high dietary N concentration could result in an imbalance between N and energy supply to the rumen and excess degradable N above requirement which consequently cause additional N excretion and environmental impact.

The increase in dietary quality (e.g. high ME) may give a better match in supplying fermentable energy and N to microbial organisms in the rumen. Fermentable energy supply, which, in case of pasture-based diets, is strongly related to herbage WSC concentrations, has been associated with advanced microbial protein synthesis and less urine N excretion because it improved ammonia utilization from rumen microbes (Tas, 2006; Dijkstra et al., 2013). Cheng et al. (2013) reported a reduction in UN/NI of non-lactating sheep as dietary N/WSC decreased. The ratio of UN/FN also decreased when WSC was added to the diet. This may explain the negative relationships between N excretion (UN and MN) and herbage WSC and ME concentrations, respectively, in the current study. On the other hand, a shift in N excretion from faeces to urine was observed by increasing N concentration and digestibility. Dong et al. (2014) demonstrated that there was a positive relationship between total tract N digestibility and the proportion of urine N in total N excretion. High N digestibility might be associated with a large proportion of N being absorbed as ammonia from the rumen, and the excess ammonia-N above requirements of microbial activity would be excreted in urine rather than in faeces, thereby increasing the proportion of N excreted in urine.

Increasing levels of NDF and ADF increased FN/NI and reduced UN/MN in the current study, which reflected the structural carbohydrates might partition more N to faeces than urine. Marini et al. (2008) found that the fermentation rate of dietary NDF has an important effect on determining endogenous N losses. High NDF concentration in cattle diet reduced the apparently digested N. The positive effect of slowly fermentable carbohydrates (NDF and ADF) on increasing N output toward faeces is possibly because they could reach the hindgut and provide an energy source for microbes that trap N, and are subsequently excreted in the faeces rather than urine (Higgs et al., 2012).

Increasing animal productivity is also a mitigation approach to reduce N excretion per unit of animal product (meat or milk) (Yan et al., 2007). In the current study, feeding level (as indicative of growth rate) was found to have a negative relationship with FN, UN and MN excretion as a proportion of NI, respectively. For example, the percentage of NI lost as UN and MN could be reduced by 14% and 18%, respectively, (Eq. 5a and 6a, Table 5) when increasing one level of feeding. Meanwhile, the ratio of UN to FN could be reduced by 37% (Eq. 7a, Table 5). This indicated that increasing feeding level could reduce more proportional N loss in urine than that in faeces. A greater efficiency of N utilization has been reported in higher yielding cows with higher proportion of milk N output and a reduction in MN excretion as a proportion of NI (Wilkerson et al., 1997). This indicates that an improvement in animal productivity can reduce the proportion of NI required for maintenance and increase the proportion of feed N incorporated into product N (milk or meat N). The dilution of maintenance requirement is a major factor for high producing animals with a low rate of N excretion per unit of NI (Yan et al., 2006). Furthermore, high feed intake can contribute to a high ruminal fractional outflow rate which leaves less time for rumen microorganisms to ferment the feedstuff, consequently lead to a reduction in ammonia-N absorbed in rumen and subsequently reduce N excreted in urine (Wischer et al., 2014).

Based on the results in the current study, increasing feeding level of good quality fresh herbage (e.g. high ME and WSC) with less N concentration has a negative effect on the partitioning of total excreted N into urine N. These findings are of specific environmental importance because the specific form in which N is excreted is important in estimating ammonia fluxes, as urinary urea is rapidly hydrolyzed to ammonium by the urease enzyme. In contrast, faecal ammonia production is generally low due to slow mineralization rates of organic nitrogenous compounds (Waldrip et al., 2013). Therefore, a switch toward more N excreted in faeces than in urine is considered desirable because of the lower volatilization of faeces N. This may consequently contribute to reduce nitrate leaching to the ground water and ammonia volatilization to atmosphere, as well as a reduction in nitrous oxide (Cheng et al., 2013; Stergiadis et al., 2015).

Conclusions

This study collected data from a wide range of fresh-cut ryegrass and animal characteristics in pasture-based sheep production system and developed a large range of prediction equations for

N excretion which give a great selection of models for use in practice, according to the availability of dietary and animal data. The equations indicated that N excretion was best predicted by dietary N intake. The accuracy of prediction could be improved when herbage chemical composition (e.g. EE, WSC and ME) and digestibility (e.g. DOMD, NDFD, ADFD and ND) were used as additional predictors. Increasing feeding level of fresh herbage with great ME concentration and low N concentration could improve N utilization efficiency and shift N excretion into faeces rather than urine. The equations developed in the current study therefore provide an approach for sheep producers to quantify N excretion against production and consequently to develop their own mitigation strategies to reduce the environment impact from sheep production systems. However, other important N-related data such as proportions of rumen degradable and undegradable protein and fermentable ME or fermentable OM supply were not assessed in the current study and they may need further investigation in predicting N excretion in sheep.

Table 1. Animal BW, herbage chemical composition, DMI, digestibility, N intake, outputs and utilization efficiency (n = 82)^{1,2}.

	Mean	SD	Min	Max
Herbage chemical composition, g/kg DM, unless otherwise stated				
DM, g/kg	155	31.3	113	237
Ash	91	18.2	57	116
GE, MJ/kg DM	18.6	0.29	18.1	19.2
N	28	7.3	13	36
NDF	499	37.9	421	594
ADF	254	17.8	209	298
WSC	156	54.0	75	292
EE	39	8.7	21	53
ME, MJ/kg DM	12.6	0.97	10.3	14.6
Digestibility, kg/kg				
DM	0.803	0.0446	0.695	0.898
N	0.729	0.0779	0.426	0.833
Digestible OM in DM	0.749	0.0424	0.654	0.845
GE	0.788	0.0472	0.681	0.883
NDF	0.800	0.0510	0.643	0.874
ADF	0.804	0.0489	0.701	0.884
BW, DMI and N intake and outputs, g/d unless otherwise stated				
BW, kg	42.9	9.61	24.5	62.7
DMI, kg/d	0.90	0.392	0.19	1.77
N intake	24.6	12.49	6.5	57.1
Faeces N	6.4	3.03	1.2	13.1
Urine N	12.6	5.91	3.0	30.1
Manure N	19.0	8.34	7.1	43.1
Retained N	5.6	5.72	-3.7	22.3
N utilization efficiency, g/g				
Faeces N/N intake	0.27	0.078	0.11	0.57
Urine N/N intake	0.54	0.178	0.24	1.11
Manure N/N intake	0.81	0.179	0.44	1.31
Retained N/N intake	0.19	0.179	-0.31	0.56
Urine N/Faeces N	2.2	1.07	0.4	7.5
Urine N/Manure N	0.66	0.100	0.30	0.88

¹ EE = ether extract; N = nitrogen; WSC = water soluble carbohydrates.

² BW = average of BW entering and BW leaving the metabolism crates.

Table 2. Correlation coefficient (r) values for relationships of herbage chemical composition, digestibility and feeding level to N utilization efficiency (g/g)^{1,2}.

Item	Faeces N/NI	Urine N/NI	Manure N/NI	Urine N/Manure N
Chemical composition, g/kg DM, unless otherwise stated				
DM, g/kg	0.32	-0.60	-0.46	-0.65
OM	0.31	-0.40	-0.26	-0.52
N	-0.49	0.44	0.23	0.65
NDF	0.29	-0.33	NS	-0.40
ADF	0.36	NS	NS	-0.27
WSC	0.32	-0.38	-0.24	-0.50
EE	-0.44	NS	NS	0.49
Energy concentration, MJ/kg DM				
GE	-0.46	NS	NS	0.39
DE	-0.71	NS	-0.26	0.46
ME	-0.54	NS	-0.42	NS
Nutrient digestibility, kg/kg				
DM digestibility	-0.66	NS	-0.32	0.35
Digestible OM in DM	-0.50	NS	-0.32	NS
N digestibility		NS	-0.22	0.77
NDF digestibility	-0.74	NS	NS	0.57
Feeding level	-0.17	-0.69	-0.76	-0.33

¹ EE = ether extract; N = nitrogen; NI = nitrogen intake; WSC = water soluble carbohydrates.

² NS = non-significant ($P > 0.05$).

Table 3. Prediction equations for N excretion (g/d) using N intake (g/d), herbage chemical composition (kg/kg DM, or MJ/kg DM) and digestibility (kg/kg) (n = 82)^{1,2}

Equations	SE	R ²	R ² _(CV)	Eq.
Faeces N = 0.12 _(0.022) NI + 3.4 _(0.90)	0.33	0.70	0.67	1a
= 0.19 _(0.017) NI + 20.5 _(7.63) ADF + 8.7 _(3.55) WSC – 1.3 _(0.13) DE + 14.2 _(3.35)	0.14	0.88	0.86	1b
= 0.22 _(0.009) NI – 20.1 _(1.05) ND + 15.6 _(0.73)	0.071	0.95	0.94	1c
= 0.21 _(0.010) NI + 62.4 _(19.73) EE + 6.7 _(2.32) WSC – 0.29 _(0.139) ME + 5.3 _(2.63) ADFD – 21.0 _(1.61) ND + 12.6 _(1.44)	0.063	0.96	0.95	1d
Urine N = 0.45 _(0.036) NI + 1.6 _(1.14)	0.99	0.77	0.75	2a
= 0.28 _(0.037) NI – 85.2 _(12.09) DM + 260.3 _(57.91) EE – 38.8 _(8.51) NDF – 28.5 _(7.83) WSC – 3.3 _(1.17) GE – 0.52 _(0.223) DE + 103.1 _(22.85)	0.39	0.69	0.68	2b
= 0.38 _(0.036) NI – 53.5 _(14.20) DOMD + 25.5 _(10.73) NDFD + 13.0 _(5.78) ND + 13.6 _(5.83)	0.89	0.82	0.80	2c
= 0.30 _(0.037) NI – 83.8 _(11.99) DM + 241.2 _(57.26) EE – 38.5 _(8.43) NDF – 28.5 _(7.81) WSC – 3.5 _(1.17) GE – 0.54 _(0.210) ME + 105.6 _(22.89)	0.39	0.69	0.68	2d
Manure N = 0.59 _(0.039) NI + 4.4 _(1.22)	1.31	0.86	0.85	3a
= 0.49 _(0.044) NI – 71.4 _(14.76) DM + 395.9 _(65.65) EE – 4.4 _(1.47) GE – 1.7 _(0.27) DE + 109.8 _(26.96)	0.61	0.82	0.81	3b
= 0.61 _(0.032) NI – 36.1 _(7.78) DOMD + 31.1 _(5.88)	1.10	0.90	0.88	3c
= 0.52 _(0.041) NI – 82.7 _(13.64) DM + 311.6 _(64.62) EE – 38.7 _(10.10) NDF – 22.5 _(8.83) WSC – 4.6 _(1.31) GE – 0.76 _(0.354) ME – 18.3 _(4.96) ND + 139.3 _(25.60)	0.49	0.80	0.80	3d

^{1,2} Values in subscript parentheses are SE; all relationships are significant ($P < 0.001$).

³ R²_(CV) = cross-validated pseudo R²

Table 4. Prediction equations for N excretion (g/d) using BW (kg), herbage chemical composition (kg/kg DM, or MJ/kg DM) and digestibility (kg/kg) (n = 82)^{1,2,3,4}.

Equations	SE	R ²	R ² _(CV)	Eq.
Faeces N = 151.0 _(27.03) EE – 1.3 _(0.17) DE + 20.0 _(2.58)	0.23	0.33	0.31	1e
= 0.08 _(0.027) BW + 192.1 _(26.98) EE – 17.1 _(1.88) ND + 8.2 _(1.90)	0.20	0.60	0.58	1f
Urine N = -74.4 _(15.24) DM + 358.0 _(61.27) EE – 44.8 _(10.49) NDF – 38.3 _(10.08) WSC + 40.5 _(9.03)	0.65	0.40	0.39	2e
= -130.9 _(21.06) DOMD + 56.8 _(14.55) NDFD + 42.4 _(7.08) ND + 34.9 _(8.37)	1.45	0.24	0.24	2f
Manure N = -69.7 _(21.10) DM + 549.7 _(85.24) EE – 45.9 _(14.99) NDF – 35.1 _(13.86) WSC – 1.8 _(0.40) DE + 65.4 _(14.72)	1.23	0.34	0.33	3e
= -70.4 _(21.91) DM + 572.5 _(86.74) EE – 87.0 _(25.65) ADF – 34.2 _(13.45) WSC – 20.1 _(5.38) ND + 52.2 _(12.88)	1.32	0.38	0.37	3f

¹ DOMD = digestible organic matter in dry matter; N = nitrogen; ND = nitrogen digestibility; NDFD = NDF digestibility; WSC = water soluble carbohydrates. The unit of DM is kg/kg.

² BW = average of BW entering and BW leaving the metabolism crates.

³ Values in subscript parentheses are SE; all relationships are significant ($P < 0.001$).

⁴ R²_(CV) = cross-validated pseudo R².

Table 5. Prediction equations for N utilization efficiency (g/g) using herbage chemical composition (kg/kg DM, or MJ/kg DM), digestibility (kg/kg) and feeding level (n = 82)^{1,2,3}.

Equations	SE	R ²	R ² _(CV)	Eq.
Faeces N = -0.069 _(0.0142) FL + 0.41 _(0.044)	0.00066	0.03	0.02	4a
/N intake = -8.9 _(1.47) N + 0.50 _(0.047)	0.00061	0.24	0.19	4b
= -8.2 _(0.91) N - 1.4 _(0.12) DOMD + 1.6 _(0.09)	0.00023	0.73	0.70	4c
= 0.95 _(0.273) ADF - 1.6 _(0.43) ASH - 0.046 _(0.0193) GE - 0.062 _(0.0052) DE + 1.9 _(0.37)	0.00023	0.75	0.70	4d
= 0.80 _(0.277) ADF - 1.8 _(0.48) ASH - 0.075 _(0.0193) GE - 0.033 _(0.0083) ME - 0.48 _(0.154) ADFD + 2.4 _(0.38)	0.00024	0.73	0.68	4e
Urine N = -0.14 _(0.017) FL + 0.81 _(0.042)	0.0026	0.47	0.44	5a
/N intake = 0.074 _(0.0168) FL ² - 0.46 _(0.074) FL + 1.1 _(0.07)	0.0022	0.58	0.54	5b
= 10.2 _(3.39) N + 0.27 _(0.101)	0.0029	0.20	0.17	5c
= -3.3 _(0.81) DM + 12.1 _(3.47) EE - 0.26 _(0.082) GE + 5.4 _(1.48)	0.0022	0.29	0.28	5d
= -4.5 _(0.90) DM + 16.5 _(4.30) EE - 5.8 _(2.41) ASH - 0.31 _(0.090) GE - 0.075 _(0.0242) ME + 1.5 _(0.44) ADFD + 6.8 _(1.76)	0.0019	0.36	0.33	5e
Manure N = -0.18 _(0.021) FL + 1.2 _(0.05)	0.0020	0.58	0.56	6a
/N intake = 0.043 _(0.0179) FL ² - 0.38 _(0.085) FL + 1.4 _(0.094)	0.0019	0.61	0.58	6b

	$= 12.1_{(4.10)}N + 0.97_{(0.476)} ADFD - 1.5_{(0.35)} ND + 0.77_{(0.272)}$	0.0028	0.23	0.16	6c
	$= -4.3_{(1.03)} DM + 16.2_{(4.96)} EE - 5.8_{(2.70)} ASH - 0.41_{(0.102)} GE - 0.050_{(0.0175)} DE + 9.8_{(2.00)}$	0.0025	0.27	0.23	6d
	$= -4.5_{(0.909)} DM + 16.4_{(4.33)} EE - 5.9_{(2.45)} ASH - 0.33_{(0.094)} GE - 0.078_{(0.0269)} ME + 1.5_{(0.48)} ADFD$ $- 0.91_{(0.315)} ND + 7.9_{(1.85)}$	0.0020	0.36	0.32	6e
Urine N	$= -0.37_{(0.153)} FL + 2.9_{(0.35)}$	0.159	0.12	0.08	7a
/Faeces N	$= 0.48_{(0.131)} FL^2 - 2.45_{(0.567)} FL + 4.8_{(0.57)}$	0.142	0.25	0.19	7b
	$= 85.6_{(20.64)} N - 0.11_{(0.594)}$	0.128	0.19	0.18	7c
	$= 87.5_{(17.83)} N + 13.5_{(2.30)} DOMD - 10.3_{(1.82)}$	0.091	0.42	0.35	7d
	$= -20.4_{(5.57)} DM + 76.8_{(26.59)} EE - 35.8_{(14.95)} ASH - 1.6_{(0.58)} GE - 0.35_{(0.166)} ME + 7.9_{(2.94)} ADFD$ $+ 6.6_{(1.94)} ND + 29.6_{(11.37)}$	0.075	0.41	0.33	7e

¹ ADFD = ADF digestibility; DOMD = digestible organic matter in dry matter; EE = ether extract; FL = feeding level = ME intake divided by ME requirement for maintenance (AFRC, 1993); N = nitrogen; ND = nitrogen digestibility; WSC = water soluble carbohydrates. The unit of DM is kg/kg.

² Values in subscript parentheses are SE; all relationships are significant ($P < 0.001$).

³ $R^2_{(CV)}$ = cross-validated pseudo R^2 .

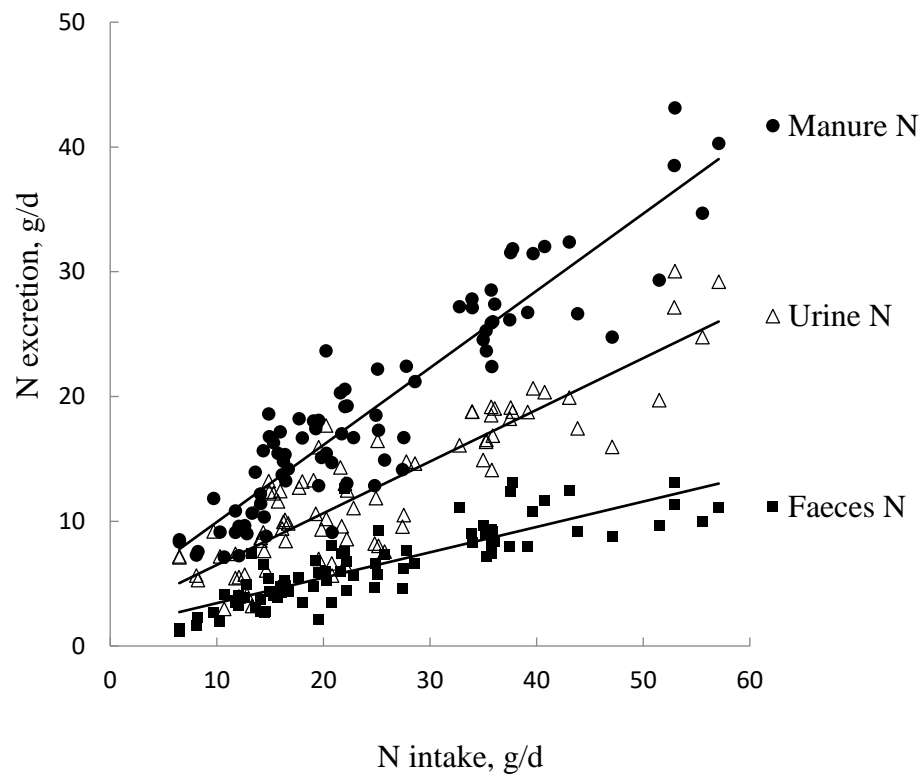


Figure 1. The relationships between nitrogen excretion and nitrogen intake (n=82)

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Appendix 1

Brief description of sheep experiments – data derived were used for the present report

S63

Effects of dietary type, breed and sex on enteric methane emissions and nitrogen utilisation efficiency in growing lambs

The objectives of the present study were to investigate the effects of dietary type, breed and sex on enteric CH₄ emissions and efficiencies of energy and N utilisation in growing lambs.

Forty-eight lowland lambs (5 months old and 36 ± 5.0 kg BW) were used in a factorial design trial with 2 breeds (Highlander vs. Texel) \times 3 sexes (female vs. intact male vs. castrated) \times 2 diets (fresh grass vs. fresh grass plus 0.5 kg/d pelleted concentrate). Treatment allocations were balanced for age and BW with 4 lambs for each breed/sex/diet combination. The 48 animals were individually housed in pens in 8 groups in sequence with 6 sheep for each time according to their schedule in chambers and fed experimental diets for 19 days, and then transferred to individual calorimeter chambers and stayed there for 5 days with measurements of feed intake, faecal and urine outputs and gaseous exchange (O₂, CO₂ and CH₄) in the final 4 d. Sheep were housed in metabolism crates individually which were placed in individual chambers. Each crate contained a feed bin, drinking water container and separate trays to collect faeces and urine. Fresh grass was harvested daily from perennial ryegrass (*Lolium perenne*) sward and offered ad libitum. Concentrate contained: barley, beet pulp, soybean meal, maize meal, molaferm and vitamin and mineral premix. Samples of grass, concentrate, urine and faeces were taken for analysis of energy and N concentrations. The DM, ash, ADF, NDF, WSC and lipid concentrations were also measured for grass, concentrate and faeces samples. Data were analysed as a 2 (breed) \times 3 (sex) \times 2 (diet) factorial arrangement using General Analysis of Variance (ANOVA) for evaluation of the effects of dietary type, breed and sex on feed intake, CH₄ emissions and N and energy utilisation.

S64

Effects of breed and dietary type on methane emissions and energy and nitrogen utilisation efficiencies in lowland replacement ewes

The objectives of the present study were to investigate the effects of dietary type and breed on enteric CH₄ emissions and efficiencies of energy and N utilisation in replacement ewes.

Sixteen replacement ewes (13 months old and 61.5 ± 5.3 kg BW) were used in a factorial design trial with 2 breeds (Highlander vs. Texel) \times 2 diets (fresh grass vs. fresh grass plus 0.5 kg/d pelleted concentrate). Treatment allocations were balanced for age and BW with 4 ewes for each breed/diet combination. Animals were individually housed in pens for 19 days, and then transferred to individual calorimeter chambers and stayed there for 5 days with measurements of feed intake, faecal and urine outputs and gaseous exchange (O_2 , CO_2 and CH_4) in the final 4 d. Sheep were housed in metabolism crates individually which were placed in individual chambers. Each crate contained a feed bin, drinking water container and separate trays to collect faeces and urine. Fresh grass was harvested daily from perennial ryegrass (*Lolium perenne*) sward and offered ad libitum. Concentrate contained: barley, beet pulp, soybean meal, maize meal, molaferm and vitamin and mineral premix. Samples of grass, concentrate, urine and faeces were taken for analysis of energy and N concentrations. The DM, ash, ADF, NDF, WSC and lipid concentrations were also measured for grass, concentrate and faeces samples. Data of CH_4 emission and energy and N utilisations were analysed using two-way analysis of variance (ANOVA) as a 2 (diet) \times 2 (breed) factorial design.

E44

Calorimetry evaluation of effects of breed and feeding level on energy metabolism and methane emissions of dry ewes offered fresh ryegrass

The objectives of the present study were to investigate the effects of sheep genotype and the level of feeding on enteric CH_4 emissions and efficiencies of energy and N utilisation in dry ewes.

Twenty-four dry ewes (16 months old and 47.6 ± 5.1 kg BW) were used in a factorial design trial with 2 breeds (Belclare vs. Lleyn) \times 3 feeding levels (1 feeding level vs. 1.5 feeding level vs. ad libitum). Treatment allocations were balanced for age and BW with 4 ewes for each feeding level/breed combination. Animals were individually housed in pens for 19 days, and then transferred to individual calorimeter chambers and stayed there for 5 days with measurements of feed intake, faecal and urine outputs and gaseous exchange (O_2 , CO_2 and CH_4) in the final 4 d. Sheep were individually housed in metabolism crates which were placed in individual chambers. Each crate contained a feed bin, drinking water container and separate trays to collect faeces and urine. All sheep were offered fresh-cut ryegrass (*Lolium perenne*) as the sole diet with 3 feeding levels. The fresh grass was harvested daily from perennial ryegrass sward. Samples of grass, concentrate, urine and faeces were taken for analysis of energy and N concentrations. The DM, ash, ADF, NDF, WSC and lipid concentrations were also measured

for grass, concentrate and faeces samples. Data of CH₄ emission and energy and N utilisations were analysed using two-way analysis of variance (ANOVA) as 3 (feeding level) × 2 (breed) factorial design.

E45

Calorimetry evaluation of effects of breed, sex and diet on energy metabolism and methane emissions of growing lambs

The objectives of the present study were to investigate the effects of sheep genotype, sex and concentrate supplement on enteric CH₄ emissions and efficiencies of energy and N utilisation in growing sheep.

Thirty-two growing lambs (5 months old and 37.8 ± 3.2 kg BW) were used in a factorial design trial with 2 breeds (Meatline vs. Suffolk) × 2 sexes (female vs. Castrated male) × 2 diets (fresh grass vs. fresh grass plus 0.5 kg/d pelleted concentrate). Treatment allocations were balanced for age and BW with 4 lambs for each breed/sex/diet combination. The 32 animals were individually housed in pens in 6 groups to go through the 6 chambers in a total of 6-weeks period. All animals were individually housed in pens and offered experimental diets for 19 days, and then transferred to calorimeter chambers and stayed there for 5 days with measurements of feed intake, faecal and urine outputs and gaseous exchange (O₂, CO₂ and CH₄) in the final 4 d. Sheep were individually housed in metabolism crates which were placed in individual chambers. Each crate contained a feed bin, drinking water container and separate trays to collect faeces and urine. Fresh grass was harvested daily from perennial ryegrass (*Lolium perenne*) sward and offered ad libitum. Concentrate contained: barley, beet pulp, soybean meal, maize meal, molaferm and vitamin and mineral premix. Samples of grass, concentrate, urine and faeces were taken for analysis of energy and N concentrations. The DM, ash, ADF, NDF, WSC and lipid concentrations were also measured for grass, concentrate and faeces samples. Data were analysed as a 2 (breed) × 2 (sex) × 2 (diet) factorial arrangement of treatments using General Analysis of Variance (ANOVA) for evaluation of the effects of dietary type, breed and sex on N and energy metabolism and CH₄ emissions.

E46

Calorimetry evaluation of effects of breeds and diets on methane emissions, energy metabolism and nitrogen utilisation of replacement ewe lambs

The objectives of the present study were to investigate the effects of sheep genotype and concentrate supplement on enteric CH₄ emissions and efficiencies of energy and N utilisation in replacement ewes.

Sixteen replacement ewe lambs (8 months old and 35.6 ± 5.2 kg BW) were used in a factorial design trial with 2 breeds (Lleny vs. Suffolk) \times 2 diets (grass silage vs. Grass silage plus 0.5 kg/d fresh concentrate). Treatment allocations were balanced for age and BW with 4 ewes for each breed/diet combination. Animals were individually housed in pens for 19 days, and then transferred to individual calorimeter chambers and stayed there for 5 days with measurements of feed intake, faecal and urine outputs and gaseous exchange (O₂, CO₂ and CH₄) in the final 4 d. Sheep were individually housed in metabolism crates which were placed in individual chambers. Each crate contained a feed bin, drinking water container and separate trays to collect faeces and urine. The grass silage was made from the secondary growth perennial ryegrass (*Lolium perenne*) sward and offered ad libitum. Concentrate contained: barley, beet pulp, soybean meal, maize meal, molaferm and vitamin and mineral premix. Samples of grass silage, concentrate, urine and faeces were taken for analysis of energy and N concentrations. The DM, ash, ADF, NDF, WSC and lipid concentrations were also measured for grass silage, concentrate and faeces samples. Data of CH₄ emission and energy and N utilisations were analysed using two-way analysis of variance (ANOVA) as a 2 (diet) \times 2 (breed) factorial design.

Appendix 2

Scientific publications achieved in the present project

Peer-reviewed scientific papers

- Wang CM, Yang CT, Zhao YG, Gilfedder T, Aubry A and Yan T. 2018. Updating maintenance energy requirement for the current sheep flocks and effects of concentrate supplement, genotype, physiological stage and sex. Submit soon to Journal of Animal Science.
- Zhao, Y.G., R. Annett and T. Yan. 2017. Effects of forage types on digestibility, methane emissions and nitrogen utilization efficiency in two genotypes of hill ewes. Journal of Animal Science 95:3762-3771. doi: 10.2527/jas.2017.1598.
- Zhao, Y.G., A. Aubry, R. Annett, N.E. O'Connell and T. Yan. 2016. Enteric methane emissions and nitrogen utilisation efficiency for two genotype of hill hoggets offered fresh, ensiled and pelleted ryegrass. Livestock Science 188: 1-8; doi: <http://dx.doi.org/10.1016/j.livsci.2016.03.016>.
- Zhao, Y.G., A.W. Gordon, N.E. O'Connell and T. Yan. 2016. Nitrogen utilisation efficiency and prediction of nitrogen excretion in sheep fed fresh perennial ryegrass (*Lolium perenne*). Journal of Animal Science 94:5321-5331, doi:10.2527/jas.2016-0541.
- Zhao, Y.G., N.E. O'Connell and T. Yan. 2016. Prediction of enteric methane emissions from sheep offered fresh perennial ryegrass (*Lolium perenne*) using data measured in indirect open-circuit respiration chambers. Journal of Animal Science, 94:2425-2435, doi: 10.2527/jas.2016-0334.
- Zhao, Y. G., A. Aubry, N.E. O'Connell, R. Annett and T. Yan. 2015. Effects of breed, sex and concentrate supplement on digestibility, enteric methane emissions and nitrogen utilization efficiency in growing lambs offered fresh grass. Journal of Animal Science 93:5764-5773; doi:10.2527/jas.2015-9515.

Scientific Conference abstracts

- Zhao, Y.G., A. Aubry and T. Yan. 2018. Development of maintenance energy requirement for sheep using calorimeter chamber data. In: Book of Abstracts of the 69th Annual Meeting of the European Federation of Animal Science, p370, Wageningen Academic Publishers, Wageningen, The Netherlands.
- Wang, C.M., Y.G. Zhou, A. Aubry, G. Arnott and T. Yan. 2018. Effects of animal breed and plane of nutrition on nutrient digestibility and energy utilisation of ewes fed fresh grass.

- In: The proceedings of the Annual Meeting of British Society of Animal Science, p174, Dublin, Ireland.
- Zhao, Y.G. and T. Yan. 2017. Effects of forage type and genotype on nutrient digestibility and energy utilisation efficiency in hill ewe lambs. In: The proceedings of the 8th European Conference on Precision Livestock Farming, P?, Nantes, France.
- Zhao, Y.G. and T. Yan. 2017. Evaluation of nitrogen utilisation efficiency in hill ewe lambs with two types of forage. In: The proceedings of the Annual Meeting of British Society of Animal Science, p91, Chester, UK.
- Zhao, Y.G., R. Annett, A. Aubry and T. Yan. 2017. Effects of forage type and genotype on methane emissions from hill ewe lambs. In: The proceedings of the Annual Meeting of British Society of Animal Science, p105, Chester, UK.
- Zhao, Y.G., A. Aubry, N.E. O'Connell and T. Yan. 2016. Effects of breed and concentrate supplementation on methane emissions from ewes fed fresh grass. In: Book of Abstracts of the 67th Annual Meeting of the European Federation of Animal Science, p469, Wageningen Academic Publishers, Wageningen, The Netherlands.
- Zhao, Y.G., A. Aubry, N.E. O'Connell and T. Yan. 2016. Effects of breed and concentrate supplementation on nitrogen utilisation in ewes fed fresh grass. In: Book of Abstracts of the 67th Annual Meeting of the European Federation of Animal Science, p458, Wageningen Academic Publishers, Wageningen, The Netherlands.
- Zhao, Y.G., N.E. O'Connell and T. Yan. 2016. Prediction of enteric methane emissions from sheep fed fresh perennial ryegrass (*Lolium perenne*) using data measured in respiration chambers. In: The proceedings of the Annual Meeting of British Society of Animal Science, p54, Chester, UK.
- Zhao, Y.G., N.E. O'Connell and T. Yan. 2016. Prediction of nitrogen excretion in sheep offered fresh-cut perennial ryegrass (*Lolium perenne*). In: The proceedings of the Annual Meeting of British Society of Animal Science, p56, Chester, UK.
- Zhao, Y.G., A. Aubry, R. Annett, N.E. O'Connell and T. Yan. 2015. Prediction of nitrogen excretion in lowland lambs offered fresh grass based diets. In: The proceedings of the Annual Meeting of British Society of Animal Science, p263, Chester, UK.
- Zhao, Y.G., A. Aubry, R. Annett, N.E. O'Connell and T. Yan. 2015. Prediction of methane emissions from lowland lambs using data measured in indirect open-circuit respiration calorimeters. In: The proceedings of the Annual Meeting of British Society of Animal Science, p142, Chester, UK.

- Zhao, Y.G., R. Annett, A. Aubry, N.E. O'Connell and T. Yan. 2014. Effects of forage and breed types on the efficiency of utilisation of energy in hill replacement ewes. In: The proceedings of the Annual Meeting of British Society of Animal Science, p24, Nottingham, UK.
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- Zhao, Y.G., A.R. McCourt, T. Yan and N.E. O'Connell. 2013. Effect of forage type on the efficiency of energy utilization in lactating dairy cows. In: The proceedings of the Annual Meeting of British Society of Animal Science, p72, Nottingham, UK.