

# Forage for CH4nge

## Experimental trial report

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### Summary of methodology

Mature ewes (n=120) were grazed in mixed-breed groups, evenly split between 3 surveyed vegetation types, after weaning of lambs. Ewes were of two breed types – Swaledale and Texel x Swaledale (crossbred) (Table 1).

Table 1: 2 x 3 trial design

	Swaledale	Crossbred
Improved grassland	20	20
Species-rich grassland	20	20
Moorland	20	20

Duplicate measurements on each ewe were taken, 2 weeks apart, using portable accumulation chambers (PAC). The first PAC measurements were taken on all ewes around 3 weeks after being on the vegetation treatments. Ewes were simultaneously gathered from the 3 grazing areas, weighed, and allocated to PAC batch, balancing for breed and vegetation treatment. Methane and carbon dioxide emissions were measured and grams per day (g/d) estimated for each ewe at each measurement event.

### Methodology

#### *Animal selection and preparation*

Animals were preselected by the farmer for measuring. Efforts were made to match animals from the 2 breed types in terms of age and other factors, and from a number of different sires / families, as far as possible. The final numbers per age group are shown in Table 2.

Table 2: Count of ewe ages by breed

Year born	Swaledale	Crossbred	Total
2019	4		4
2020	2	16	18
2021	20	9	29
2022	18	12	30
2023	16	12	28

2024	7	7
older	4	4
<b>Grand Total</b>	<b>60</b>	<b>120</b>

Ewes were allocated to grazing treatment in late August, after weaning of lambs, balancing for breed and ewe age, as far as possible. The three forage treatments (grazing areas) were improved grassland, species-rich grassland and moor, with one field / grazing area per treatment, which were surveyed in summer to identify species of plants included in the available vegetation.

A list of ewe identification numbers (EID), with their breed, forage-treatment, age, live weight and body condition score (taken by farm staff at the time of going into the treatment grazing areas) was used to allocate ewes into batches of 12 for PAC measurements (round 1 and round 2 – ewes reallocated between rounds), balancing these factors across batches, as far as possible. In advance of PAC measurements, sheep were sprayed to identify them into a half-day session (day1 or day2, morning or afternoon) to enable gathering only the ewes for each session into the pens on the day.

Animals remained on pasture undisturbed for ~3 weeks prior to the first PAC measurement, to maximise the opportunity for normal levels of feed intake. Biomass measurements were taken of improved grassland and species-rich grazing areas (fields) the day before PAC measurements. This was not practical for the moor grazing area, due to the size and heterogeneity of the grazing area.

### *PAC measurements*

Animals were only taken off feed on the day of PAC, at the start of their session. All animals for a session were removed from pasture at least 1 hour before the planned start of PAC measurements. Although efforts were made to reduce differences in time that ewes were taken off the 3 treatment fields, in reality these differed by as much as 1.5 hours within a session (order that fields were gathered differed between sessions). These timings were recorded to allow this information to be fitted in the analysis models. Time off feed prior to PAC, for each individual sheep, ranged from 1-5 hours. All animals were weighed at the start of the session and drafted into their allocated batches of 12 for testing. Two ewes of each breed x forage-treatment combination were included in each batch of 12, to allow comparisons within batch.

### *Gas measurements*

Methane, oxygen and CO<sub>2</sub> were measured on entry (time 0) and at approximately 25 and 50 minutes after confinement in the sealed chambers. The gaseous measurements of methane obtained for each animal over each measurement run were converted to litres/day (l/day) using an equation to account for time and methane measurement differences, chamber volume and live weight (Jonker *et al.*, 2018; O'Connor *et al.*, 2021).. A similar equation was used to convert the O<sub>2</sub> and CO<sub>2</sub> measurements obtained for each animal over each

measurement period to litres per day. The final gas volumes obtained in l/day were then extrapolated up to g/day values using an equation accounting for pressure, temperature and molecular weights of the gases (Jonker *et al.*, 2018; O'Connor *et al.*, 2021).

To account for variation between batches in time off feed, handling, environmental conditions etc., the methane and CO<sub>2</sub> measurements (g/d) for each animal were adjusted for batch mean and rescaled to the population mean (mean of all ewes measured per visit). These rescaled values (**CH4\_resc** and **CO2\_resc**) were used as the final methane and CO<sub>2</sub> values for each animal per visit.

### *Statistical analysis*

The full data set, including the two rounds of PAC data, consisted of 238 records in total. A Generalised Linear Mixed Model was fitted in Genstat (24<sup>th</sup> edition):

**Model 1:** CH4\_resc = Round + Unq\_lot + MinsOffFeed + EweAge + Breed + (Round x Forage\_treatment) + EID

Where: Round (1 or 2) accounted for first or second measurement; Unq\_lot was batch of 12 that the ewe was measured in (20 levels); MinsOffFeed was the number of minutes between the ewe being gathered from the field and being recorded in PAC; EweAge was the age of the ewe in years (1 - 6); Breed was Swaledale or crossbred; Forage\_treatment was improved grassland, species-rich grassland or moor. All previous terms were fitted as fixed effects. EID was a random effect to account for repeated measures from the same animal.

All single or interaction terms between Round, Breed and Forage\_treatment were tested in model, but only significant terms were retained for analysis.

The same maximum model was then tested for CH4\_resc with live weight (PACLWT) included in the model. The final model, after dropping non-significant terms (except breed), included:

**Model 2:** CH4\_resc = PACLWT + Round + Unq\_Lot + EweAge + Forage\_treatment + Breed + EID

A similar model was run to investigate significant effects on CO<sub>2</sub>\_resc, with the final model, after dropping non-significant terms, including:

**Model 3:** CO2\_resc = PACLWT + Round + EweAge + Breed + Forage\_treatment + (Round x Forage\_treatment) + EID

## **Results**

### *Ewe weights and condition scores*

Table 3: Average live weights in kg (LW0) and body condition scores (BCS0) at the start of the grazing trial, and changes in live weights to the first PAC visit (LW1) and second PAC visit (LW2)

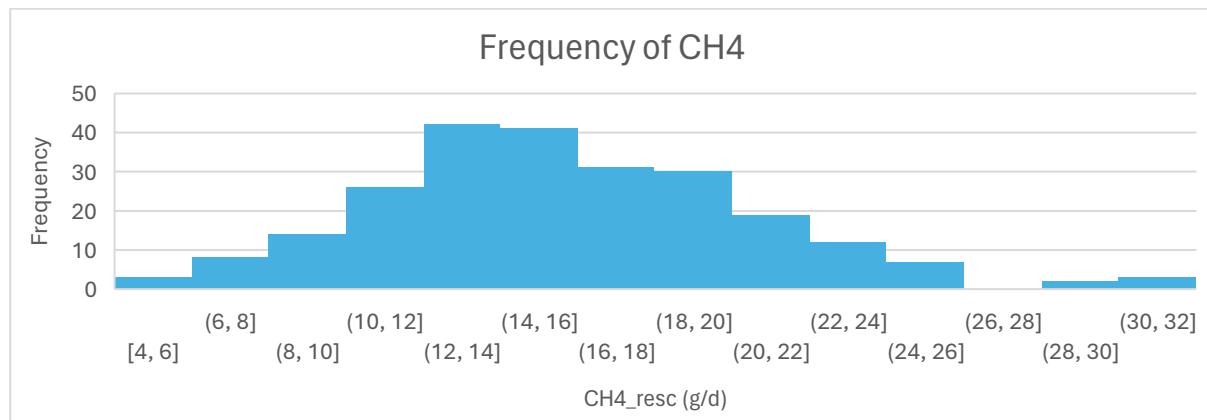
Breed	Forage treatment	LW0	BCS0	LW1-LW0	LW2-LW1
Swaledale	Improved grassland	58.4	2.16	-2.2	0.9
	Species-rich grassland	56.9	2.10	-1.4	0.6
	Moor	59.6	2.31	-3.6	0.9
	<b>Total</b>	58.3	2.19	-2.4	0.8
Crossbred	Improved grassland	77.4	3.40	-2.2	-0.2
	Species-rich grassland	77.4	3.46	-3.6	0.4
	Moor	77.3	3.63	-7.0	1.1
	<b>Total</b>	77.3	3.50	-4.2	0.4
<b>Grand Total</b>		67.9	2.84	-3.3	0.6

At the start of the grazing trial, the Swaledale ewes were lighter on average by 19kg (Table 3), with live weights ranging from 44-74kg, compared to crossbred ewes that ranged from 60-99kg. The crossbred ewes also had higher body condition score by 1.31 units on average. Ewes lost weight between the start of the grazing trial and the first PAC measurement, with Swaledale ewes losing less on average (2.4kg, 4.1%) than crossbred ewes (4.2kg, 5.4%). Between the two PAC measurements, ewes gained weight on average, except the crossbred ewes on the improved grassland (which lost 0.2kg on average), but this group had lost less weight than the other crossbred groups before the first PAC measurement.

#### *CH4 emissions*

The distribution of raw CH4\_resc values across the two rounds of PAC (n=238) is shown in Figure 1. Values ranged from 4.38 – 31.98 g/d.

Figure 1: Distribution of CH4 values



After adjusting for the terms included in model 1, Swaledale ewes were found to produce significantly less methane per day per animal than crossbred ewes, on average (14.6 vs 17.1 g/d; P<0.05). However, after adjusting for live weight (model 2) there was no significant breed

difference in methane emissions, suggesting that any breed differences observed were only due to differences in body size.

After adjusting for live weight, average methane emissions differed significantly ( $P<0.05$ ) due to round (1>2), lot (batch of 12 animals in 1 PAC run), and ewe age (younger age groups of ewes generally producing more methane; Table 4).

Table 4: Means for CH4\_resc for each ewe age

Ewe age	Mean CH4
1	17.35
2	16.75
3	17.15
4	15.76
5	13.54
6	15.14

Methane differences due to forage type were significant: ewes on improved grassland produced significantly more methane than those on species-rich grassland or moor (Figure 2). Forage availability data for the improved and species-rich grasslands are shown in Table 5. No grass height or biomass data were available for the moor.

Figure 2: Mean methane emissions from ewes on each forage-treatment (means sharing a common superscript are not significantly different,  $P>0.05$ )

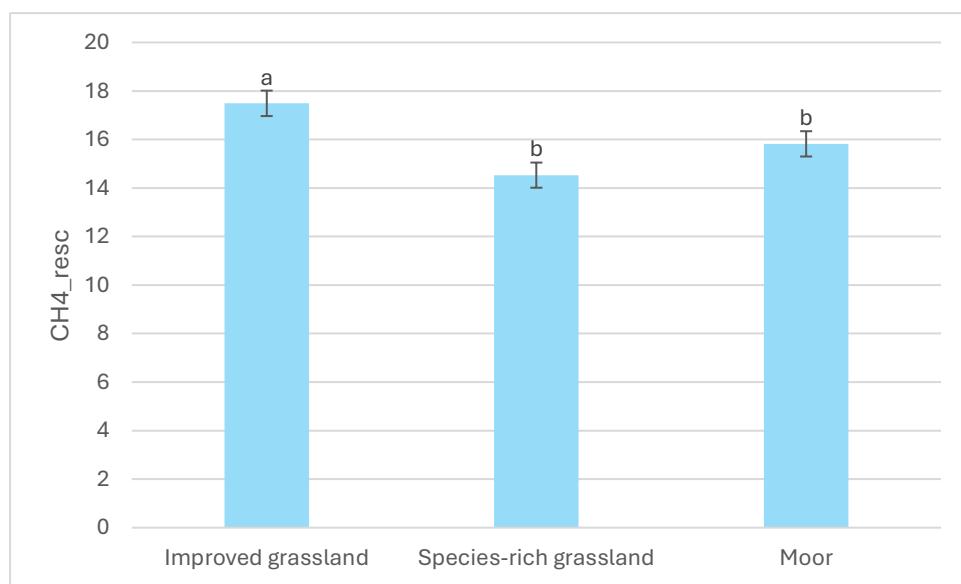


Table 5: Forage availability data taken prior to each PAC measurement.

Species-rich grassland		Improved grassland	
Grass height (cm)	Biomass (kg DM/ha)	Grass height (cm)	Biomass (kg DM/ha)

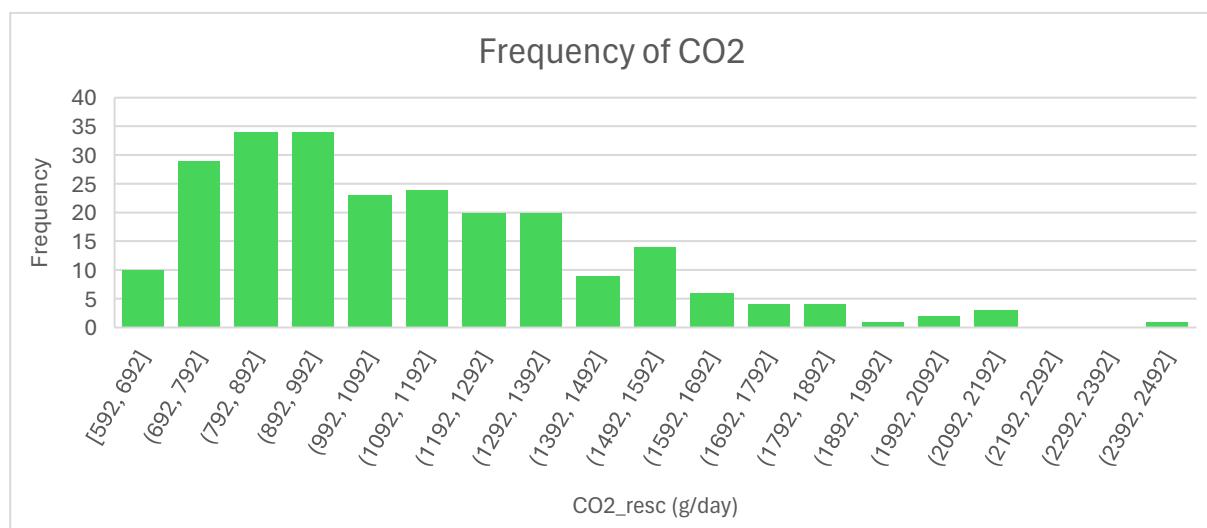
Round 1	14.3	2502	14.1	2474
Round 2	11.0	2040	15.3	2642

No significant breed x forage interaction was observed (P>0.05).

### CO2 emissions

The distribution of raw CO2\_resc values across the two rounds of PAC (n=238) is shown in Figure 3. Values ranged from 592 – 2473 g/d and are skewed to the left.

Figure 3: Distribution of CO2 values



After adjusting for the terms included in model 3 (including live weight), Swaledale ewes were found to produce significantly less CO2 per day per animal than crossbred ewes, on average (979 vs 1195 g/d). Since live weight was included in the model, this suggests that the difference seen between breeds were not due to differences in body size.

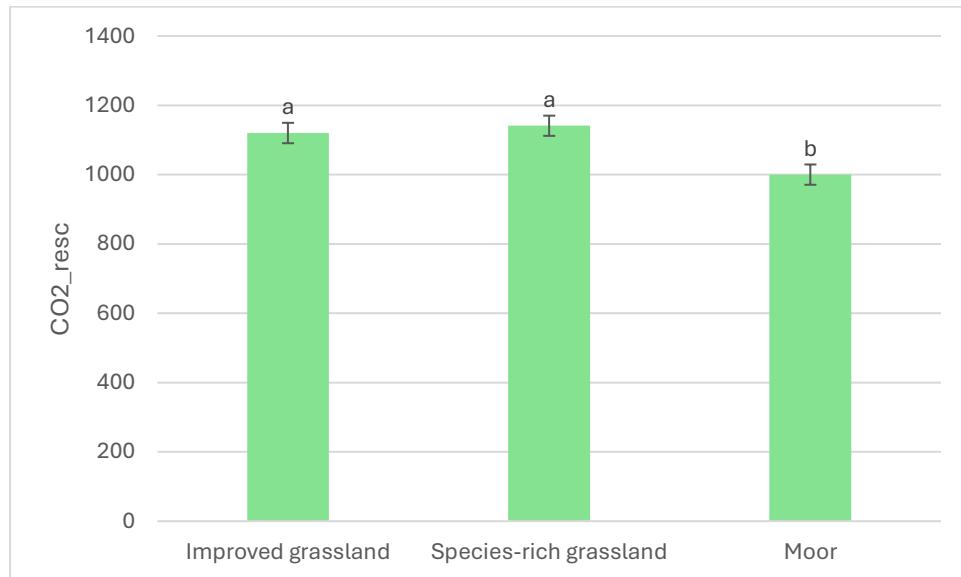
Average CO2 emissions differed significantly (P<0.05) due to live weight, ewe age (middle age groups of ewes generally producing more CO2; Table 6), breed, forage treatment and forage treatment x round interaction.

Table 6: Means for CO2\_resc for each ewe age

Ewe age	Mean CO2
1	1067
2	1132
3	1132
4	1175
5	1062
6	953

CO<sub>2</sub> differences due to forage type were significant: ewes on improved grassland and species-rich grassland produced significantly more CO<sub>2</sub> than those on the moor (Figure 2).

Figure 2: Mean CO<sub>2</sub> emissions from ewes on each forage-treatment (means sharing a common superscript are not significantly different, P>0.05)



No significant breed x forage interaction was observed (P>0.05), however, there was a significant forage x round effect (P<0.05), meaning that the comparison between forage treatments, in terms of ewe CO<sub>2</sub> emissions, differed between rounds 1 and 2 of measurement (Table 7). In round 1, the CO<sub>2</sub> from ewes on the species-rich grassland was significantly higher than those on the moor, with CO<sub>2</sub> from ewes on the improved grassland intermediate and not significantly different from either of the other treatments. However, in round 2, the CO<sub>2</sub> emissions from ewes on the moor were significantly lower than the ewes in either of the other 2 forage treatments, which were not significantly different to each other.

Table 7: Mean CO<sub>2</sub> emissions for ewes grazing each forage type at each PAC visit

Round	Improved grassland	Species-rich grassland	Moor
visit 1	1092 <sup>ab</sup>	1143 <sup>a</sup>	1043 <sup>b</sup>
visit 2	1148 <sup>a</sup>	1138 <sup>a</sup>	958 <sup>c</sup>

Means sharing a common superscript are not significantly different (P>0.05).

## Conclusions

In conclusion, large amounts of variation were observed in both CH<sub>4</sub> and CO<sub>2</sub> across and within breeds. Swaledale sheep produced less methane than Texel x Swaledale ewes, but

once adjusted for differences in live weight, no significant breed differences were observed. However, even after accounting for differences in live weight, Swaledale ewes produced less CO<sub>2</sub> than comparable crossbred ewes.

Ewes grazing on improved grassland produced more methane and CO<sub>2</sub> than those grazing on the moor. Ewes grazing the species-rich grassland produced similar levels of methane as those on the moor, but similar CO<sub>2</sub> as those on the improved grassland. These differences between forage types may be associated with forage availability or dry matter intake, which cannot be fully assessed here.

### **Reference List**

Jonker A, Hickey S, Rowe S, Janssen P, Shackell G, Elmes S, Bain W, Wing J, Greer G, Bryson B, MacLean S, Dodds K, Pinares-Patino C, Young E, Knowler K, Pickering N and McEwan J 7-5-2018. Genetic parameters of methane emissions determined using portable accumulation chambers in lambs and ewes grazing pasture and genetic correlations with emissions determined in respiration chambers. *Journal of Animal Science* 96, 3031-3042.

O'Connor E, McHugh N, Boland TM, Dunne E and McGovern FM 1-8-2021. Investigation of intra-day variability of gaseous measurements in sheep using portable accumulation chambers. *Journal of Animal Science* 99, 1-10.