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Performance and enteric methane emissions from housed beef cattle fed silage produced on pastures with different forage profiles

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ABSTRACT

Methane (**CH_4**) produced by ruminants is a significant source of greenhouse gases from agriculture in the United Kingdom (**UK**), accounting for approximately 50% of the emissions in this sector. Ration modification is linked to changes in rumen fermentation and can be an effective means of CH_4 abatement. In temperate climate countries, forage silage represents a major feed component for cattle during the housing period. The objective of this study was, therefore, to compare enteric CH_4 emission from cattle offered silage produced from different types of grassland. Beef cattle, steers (n = 89) and heifers (n = 88) with average liveweight (**LW**) of 328 ± 57.1 kg were evaluated during two housing seasons (2016–2017 and 2017–2018) from November to April, at the Rothamsted Research North Wyke Farm Platform (UK). The treatments corresponded to three diet types, comprising silage harvested from three different pastures: **MRG**, monoculture of perennial ryegrass (**PRG**, *Lolium perenne* L.cv. AberMagic), bred to express the high-sugar phenotype; **RG-WC**, a mixed sward comprised of the same perennial ryegrass cultivar with white clover (*Trifolium repens* L.) with a target clover proportion of 30% as land cover; and permanent pasture (**PP**) dominated by PRG and a small number of non-introduced species. MRG and PP received 160–200 kg N/ha/year. Cattle were weighed every 30 days, and the enteric CH_4 emission was determined using GreenFeed automated systems. No significant differences in enteric CH_4 emission per head or per kg LW were observed between treatments. However, emission expressed per average daily gain (**ADG**) in LW was greater ($P < 0.001$) for MRG compared with RG-WC and PP, at 270, 248 and 235 g CH_4/kg ADG, respectively. This related to a lower ADG ($P = 0.041$) for the animals fed MRG silage compared with RG-WC and PP which were similar, with respective values of 0.67, 0.71 and 0.74 kg/day. The forages compared in this study showed little or no potential to reduce enteric CH_4 emission when fed as silage to growing beef cattle during the winter housing period.

Implications

Understanding the impact of different ensiled forages on beef cattle performance and enteric methane emissions during housing contributes to the development of potential, feasible and practical measures that producers can take towards more sustainable beef production systems. The nutritional benefits of improved ryegrass species when freshly grazed may not be apparent following the ensiling process. Optimum ensiling practices and careful choice of forage species should be adopted in seeking to improve growing beef cattle performance and reduce enteric methane emission intensity.

Introduction

Ruminant production makes an important contribution globally to human food, in particular the conversion of human non-edible protein (e.g. forages) to human edible protein (i.e. milk and meat) (Wilkinson and Lee, 2018). However, enteric methane (**CH_4**) produced by ruminants accounts for approximately 6% of global anthropogenic greenhouse gas (**GHG**) emissions (Beauchemin et al., 2020), and 50% of GHG emissions from United Kingdom (**UK**) agriculture (Brown et al., 2020). With increasing global demand for meat and milk, which is expected to increase by 73 and 58%, respectively, by 2050 compared with 2010 (Beauchemin

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et al., 2020), the pressure to develop more sustainable production systems will continue to increase.

Methane is a short-lived climate pollutant, with an average lifetime in the atmosphere of 13.5 years (Doble & Kruthiventi, 2007). This creates an opportunity for short-term gains in abating global warming by focusing on reductions in CH_4 emissions (Lynch et al., 2020), particularly in forage-based systems which may also give an opportunity for carbon sequestration (Beauchemin et al., 2020). Management practices such as the timing of harvest, the use of improved varieties/species with higher quality and digestibility, and ensiling techniques aimed at conserving the digestible nutrient content may all help in this respect (McGee, 2005).

Enteric CH_4 production is influenced by the substrates (predominately carbohydrate) available from the animal's diet and the subsequent fermentative profile that develops through the action of the rumen microbial population (Roque et al., 2021). A forage-based diet is rich in structural carbohydrates such as cellulose, hemi-cellulose and lignin; as the amount of these increases, the pH in the rumen also increases, leading to greater production of acetate over propionate. The production of acetate is accompanied by the production of H^+ which acts as a substrate for methanogenesis in the rumen, whereas propionate is a H^+ sink. The higher pH and greater release of H^+ in the rumen therefore benefit methanogenic populations (Haque, 2018). An effective way of prioritizing the propionate pathway to consume H^+, rather than being used for methanogenesis, is the inclusion of cereal-based concentrates in the diet, which are rich in non-structural carbohydrates such as starch and sugar which leads to a decrease in the rumen pH and favours propionate formation (Beauchemin et al., 2020). However, grass silage is the basic component of many beef production systems globally, particularly in countries with temperate climates such as northern and western Europe and provides the main source of fibre when the animals are housed during the indoor/winter period (McGee, 2005).

The aim of the present study was to assess the potential, within forage-based diets, to similarly reduce enteric CH_4 through differences in forage carbohydrate form and/or active plant secondary metabolites (Archimède et al., 2011). Specifically, the Rothamsted Research North Wyke Farm Platform (**NWFP**) was used to test the hypotheses that, compared to silage made from a permanent pasture of predominantly perennial ryegrass: (1) silage made from ryegrass (**MRG**) bred to express high water-soluble carbohydrate (**WSC**) content, and (2) silage made from a mixed sward containing the higher WSC ryegrass and white clover, would reduce enteric CH_4 emissions and/or increase animal productivity from housed beef steers and heifers.

Material and methods

Facilities and treatments

The trial was conducted on the NWFP, in the southwest of England 50.7765° N 3.9235° W at an altitude of 154 m. The climate is temperate, oceanic (type Cfb in the Köppen-Geiger classification), with a mean annual temperature and rainfall of 9.3 °C and 1040 mm, respectively. Details of the NWFP are provided by Orr et al. (2016), Takahashi et al. (2018), and Lee et al. (2021), but, briefly, at the time of this trial, it comprised three farmlets each of approximately 21 ha and each supporting 30 growing beef cattle (Stabilizer, Stabilizer cross, Charolais cross and Limousin cross) and 75 Suffolk × Mule ewes with their lambs sired by Charolais rams.

The animals were randomly allocated to the three farmlets using a covariate-based constrained randomisation, stratified by breed and gender and taking account of growth rate, weaning weight and age to ensure the three groups of animals were similar in these characteristics. The farmlets differed in terms of the forage being grown, being either: (i) permanent pasture (**PP**), predominantly perennial ryegrass (*Lolium perenne*) but with a mixture of naturalised species; (ii) reseeded monoculture ryegrass (MRG) bred to express high water-soluble carbohydrate (WSC) content (*Lolium perenne* cv. AberMagic); (iii) reseeded mixture of perennial ryegrass (*Lolium perenne* cv. AberMagic) alongside white clover (*Trifolium repens* cv. AberHerald) (**RG-WC**), with a target clover proportion of 30% of the land cover. At the time of this study, the PP farmlet had been established for about 30 years. Farmlets MRG and RG-WC were established between 2013 and 2015 (one third of the farmlet in each year) by spraying glyphosate to eliminate the existing permanent pasture, followed by ploughing, cultivation, and planting in July and August of each year.

The PP and MRG received up to 200 kg N fertiliser/ha/year while the RG-WC farmlet received up to 40 kg N fertiliser/ha/year. The three farmlets were fertilized with P, K and S prior to forage cutting, and received lime as required if soil pH was below 6. For each treatment, the farmyard manure produced during the animal housing period was applied to fields within that farmlet following silage harvest. Forage on each farmlet was managed for grazing by both the cattle and sheep and to produce conserved silage for feeding the animals for the housed period (Takahashi et al., 2018). Silage harvest is typically carried out in early May (first cut) and July/August (second cut), depending on the weather conditions. The first cut is stored in clamps and the second as silage bales, both treated with a bacterial silage inoculant (MoleActive, Mole Valley Farmers, South Molton, Devon, UK) composed of bacteria (*pediococcus pentosaceus, lactobacillus plantarum, lactobacillus brevis*), enzymes (xylanase and cellulase), and nutritional additives (dextrose, manganese sulphate and anti-caking agent silicone dioxide); 150 g of inoculant were added every 50 tonnes of fresh forage mass.

During the housed period, the beef cattle were kept in three identical adjacent but detached barns (one for each farmlet), each following the same design and orientation (East-West) and constructed specifically for the NWFP. The barns were 48 × 15 m internally, including a 4 × 48 m walk/tractor way that cattle had no access to. The study group had half of the available space (24 × 11 m) which included a bedding area (7.5 × 24 m) and feeding/drinking area (4.5 × 24 m), where the feed and water were offered ad libitum.

Bedding of the animal pens was carried out every morning using a tractor-trailed straw chopper. The feeding passage was also scraped every morning to remove dung and urine using a tractor-mounted yard scraper. The diet comprised the silage specific to each farmlet, together with a mineral mix (Cattle Min GP IF, Feedco Ltd, Exeter, Devon, UK) included at 3 g/kg of DM. The ration was distributed along the length of the feed passage using a forage mixer wagon every morning after bedding and scraping. Every evening, any remaining feed was pushed up to ensure it could be reached by the animals. The forage mixer wagon was equipped with weigh cells enabling measurement of the offered silage amount per feeding.

Forage analyses

Samples of the diet as fed were collected once per week, with 300 g taken from five different points in the feed passage and stored at −20 °C prior to subsequent analysis. For chemical analysis, the samples were freeze dried (Multidrier, Frozen in Time Ltd., York, North Yorkshire, UK) at −20 °C for fourteen days, with DM content (%) being determined from the loss in weight. After drying, samples were milled through a 0.5 mm screen for CP determination and a 2 mm screen for fibre fraction content analysis.

The determination of the content (%) of NDF, ADF, modified acid detergent fibre (**MADF**), and ADL were conducted in an ANKOM 2000® automated fibre analyser (ANKOM Technology, Macedon, NY, USA), following, respectively, the Ankom methods 1, 2, and 3. The water-soluble carbohydrate content (**WSC**, %) was quantified through HPLC (1260 Infinity II, Agilent Technologies, Didcot, Oxfordshire, UK) according to Johansen et al. (1996). For the determination of CP content, the total N content was quantified using a Carlo Erba NA 2000 element analyser (CE Instruments Ltc, Wigan, UK) linked with a Sercon 20:22 isotope ratio mass spectrometer (Sercon Ltd., Crewe, UK) and then multiplied by the constant 6.25. Ash content was determined by burning in a muffle furnace (CWF 1100, Carbolite Gero Ltd., Hope, Derbyshire, UK) at 550 °C for 4 hours. Metabolisable energy content (**ME**, MJ/kg DM) was calculated based on the equation proposed by Givens et al. (1989).

$$ME = 15.0 - 0.014 \times MADF$$

Parameters on the quality of the silage fermentation (lactic acid, VFA, ammonia-N, pH) were not determined.

Animal performance and enteric methane emissions

To monitor changes in live weight (**LW**) and to calculate the average daily gain (**ADG**), the cattle were weighed once a month during the experimental period.

Measurement data for enteric CH_4 emissions were collected from individual beef cattle (Stabilizer, Stabilizer cross, Charolais cross and Limousin cross) from the Rothamsted Research – North Wyke farm experimental herd during the winter housing periods (November to April) of 2016/2017 and 2017/2018. The cattle enter the farmlets at the start of the winter housing period (typically October/November) as weaned animals of approximately 7 months of age. From the Spring of the following year, they go out to grazing on their respective farmlets and, as far as possible, are reared to their finishing weight while at grazing. Some animals require a second housing period to reach the required finishing weight (c.600–625 kg) but enteric CH_4 emissions were not made from any cattle during either the grazing or second winter housing period. Two cohorts of cattle were therefore followed across the two study years, each during their first winter housing period, with measurements from a total of 177 individual animals (89 steers and 88 heifers). Average age and LW of the cattle entering the first housed period were 220 ± 31.3 days and 328 ± 57.1 kg, respectively.

The enteric CH_4 emissions were determined using three GreenFeed (**GF**) units (C-lock Inc., Rapid City, SD, USA) as described by Huhtanen et al. (2015), and Della Rosa et al. (2021). This automated system measures gas fluxes from individual animals when they voluntarily visit the equipment and are rewarded with a small quantity of pelleted feed. The air exhaled through the mouth and nostrils of the animal is aspirated, filtered, and then analysed in real-time by a non-dispersive infrared sensor, while the flow of the aspirated air is also determined by a flowmeter. Average daily emissions are estimated by combining data from multiple visits over the observation period.

Each group of thirty animals had access to one GF unit throughout the first winter housing period, which lasted 159 days in the first year (04/11/2016–12/04/2017) and 167 days in the second year (08/11/2017–24/04/2018). The first fourteen days were considered as an adaptation period, and data from these were discounted. A commercial pelleted feed ("Super Rearer 18", ForFarmers, Rougham, Bury St Edmunds, UK) was used as an attractant in the GF units, with the composition of 86.5% DM, 18.0% CP, 10.6% fibre, 8.3% Ash, 5.3% ether extract, 1.6% calcium, and 0.6% phosphorus.

The GF units were programmed to permit up to five feeding periods (**FPs**) per animal per day, with a minimum interval of 0440 h between each. For every FP, a maximum of five feed drops each containing an average of 30 g of pellets were allowed, with an interval of 35 s between drops. Average daily concentrate consumption per treatment group was 450, 435, and 520 g for RG-WC, PP and MRG, respectively, based on respective average recorded daily drop numbers of 15.0, 14.5 and 17.3.

The start of a FP was triggered by an animal inserting its head into the GF, at which point the animal was identified by its radio frequency identification (**RFID**) ear tag, the first feed drop initiated and measurement of CH_4 and CO_2 from expiration and eructation commenced. Once the animal left, feed drops would stop being dispensed and gas measurement stopped. Across the study period, 42 950 individual FPs were recorded, with an equivalent number of enteric CH_4 samples.

Standard gas calibrations using a mix of CH_4 (508 ppm) and CO_2 (4 982 ppm) in zero-grade nitrogen were performed automatically every day at 0400 h and once per month, a CO_2 recovery calibration was performed. Air filters were checked, changed, and cleaned on a weekly basis. Details on the calculation procedure for CH_4 and CO_2 emission are described by Martin et al. (2020).

Statistical analyses

Silage chemical characteristics (CP, WSC, NDF, ADF, MADF, ADL, Ash and ME contents) were analysed using ANOVA with a factorial treatment structure (farmlet.year) and sampling timepoints treated as blocks (20 sampling times per year = 40 blocks total). The effect of year was tested between blocks, and farmlet was tested within blocks as was their interaction. The observations for WSC, ADL and Ash were $\log_e$ transformed in order to satisfy the normality and equal variance assumptions of the analysis.

A linear mixed model was used to assess the effects of farmlet (i.e. silage), and year, on the observations of initial liveweight (**ILW**), final liveweight (**FLW**), ADG, CO_2 per animal, enteric CH_4 per animal, and enteric CH_4 in relation to the ADG and LW while taking into account cattle breed and gender. The fixed structure for the model included farmlet and farmlet.year and the random structure included breed, gender and animal (Breed.Sex/Farm_Number). The data were averaged to a single observation per animal within each year prior to this analysis and one animal was excluded due to a much lower number of visits than the others. No transformations were required other than for CH_4/ADG which was $\log_e$ transformed. The treatment effect was considered as significant at $P < 0.05$. This was determined based on the F tests produced when fitting the linear mixed model. All analyses were carried out using Genstat 21.

Results

Forage analyses

There were significant effects of farmlet, year, and farmlet.year interaction for the silage DM concentration (Table 1), with MRG silage having a lower average DM concentration across the two years than PP or RG-WC. The average silage DM concentration across farmlets was higher in the first year than the second year, and there were no significant differences between farmlets in the second year (Table 1). Silage from the PP farmlet had the highest CP concentration, followed by that from MRG, with RG-WC being the lowest (Table 1). There was a farmlet.year interaction for CP concentration, with greater CP concentration for PP compared with MRG and RG-WC in the first year, and greater CP concentration in PP and MRG compared with RG-WC in the second year (Supplementary Table S1).

Table 1
Performance and emissions of beef cattle fed with silage produced in farmlets with different types of forage, silage chemical characteristics, effects of farmlet, year and interaction farmlet.year.

Response	Treatment			Average		*P*-value		
	RG-WC	PP	MRG	Year 1	Year 2	Farmlet	Year	Farmlet.Year
DM (%)	31.3[a]	33.1[a]	27.9[b]	33.2[a]	28.4[b]	<0.001	<0.001	0.005
CP (% of DM)	12.4[c]	15.8[a]	13.8[b]	13.9	13.8	<0.001	0.666	0.004
WSC (% of DM)[1]	1.83 (6.2)[a]	1.14 (3.1)[b]	0.84 (2.3)[c]	1.37[a]	1.15[b]	<0.001	0.023	<0.001
NDF (% of DM)	44.0[b]	48.2[a]	47.2[a]	48.7[a]	44.2[b]	<0.001	<0.001	0.061
ADF (% of DM)	26.4[b]	27.4[a]	28.0[a]	28.4[a]	26.1[b]	0.004	<0.001	0.024
MADF (% of DM)	29.0[b]	29.7[a]	30.4[a]	30.2[a]	29.2[b]	0.002	<0.001	<0.001
ADL (% of DM)[1]	1.12 (3.1)	1.11 (3.0)	1.09 (3.0)	1.25[a]	0.96[b]	0.832	0.002	0.025
ME (MJ per kg DM)	10.9[a]	10.9[b]	10.8[b]	10.8[b]	10.9[a]	0.002	<0.001	<0.001
Ash (% of DM)[1]	2.15 (8.5)	2.16 (8.7)	2.16 (8.7)	2.09[b]	2.21[a]	0.796	<0.001	0.221

Abbreviations: RG-WC = Mixed sward; PP = Permanent pasture; MRG = Reseeded monoculture ryegrass; WSC = Water-soluble carbohydrates; MADF = Modified acid detergent; ME = Metabolisable energy.
[1] Log_e numbers in brackets are the back-transformed means.
[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$.

There was a significant effect of farmlet on the silage WSC concentration, averaged across years and within each individual year (Table 1), with silage WSC concentration in the order RG-WC > PP > MRG. The interaction farmlet.year was significant due to a higher WSC concentration in the first year for the RG-WC treatment, while the others did not differ, while in the second year, RG-WC > PP > MRG (Supplementary Table S1). Silage NDF concentration was significantly lower for RG-WC than the other two farmlets, which did not differ from each other. NDF was also significantly greater in the first year. There was a significant difference between farmlets in silage ADF and MADF concentration, with values for MRG and PP being greater than for RG-WC. Average silage ADF and MADF concentrations were greater in year 1 than year 2. There was also an interaction between farmlet and year for silage ADF concentration, with that for MRG being greater than PP and RG-WC in year 1, and RG-WC being significantly lower than MRG and PP in year 2. For MADF, MRG silage had a significantly greater concentration than PP and RG-WC in year 1, while in year 2, the values were in the order PP > MRG > RG-WC.

Average silage ADL concentration was greater in the first year compared to the second, and there was a significant farmlet.year interaction, with greater ADL concentration for PP and RG-WC than MRG treatment in the first year and greater for MRG compared with PP (with RG-WC being not significantly different to either) in the second year (Table 1). Silage ME concentration was greater for RG-WC than MRG, with that for PP silage being intermediate between these. Silage ME concentration was greater in year 2. There was a significant effect of year, but not farmlet and no significant farmlet.year interaction for silage ash concentration, with ash being higher in the second year compared to the first.

Animal performance and emission

Average cattle ILW and FLW did not differ significantly between farmlets, but average liveweights were greater in the second year compared to the first (Table 2). There was a significant difference between farmlets in ADG where the animals receiving the PP silage performed better than the animals fed with MRG silage, while the cattle on RG-WC treatment did not differ from either. Cattle ADG was also significantly greater in year two (Table 2).

Daily CO_2 emission per animal was greater in the first year than in the second (Table 2). In the first year, daily CO_2 emission was greater for PP than RG-WC, while MRG did not differ significantly from the other two. In contrast, in the second year, the daily CO_2 emission were lower for PP than those on the MRG and RG-WC, which did not differ from each other. Overall, there was no significant difference between farmlets or year in enteric CH_4 emissions per animal, although in the second year, daily CH_4 emission per head was significantly lower for PP when compared with the other two farmlets (Table 2).

There was a significant farmlet.year interaction on CH_4 emission expressed as a function of ADG, with higher emission per ADG for MRG compared with PP, while RG-WC did not differ from the other two treatments (Supplementary Table S2). Average CH_4 emission per ADG was greater in the first year compared to the second. There was also a farmlet.year interaction, with greater emission per ADG for MRG compared with RG-WC and PP in the first year, and greater emission per ADG for RG-WC compared to PP in the second year with MRG not significantly different from either (Supplementary Table S2). There was no significant effect of farmlet on CH_4 emission per unit LW (Table 2). However, there was a year effect, with greater emission per LW in the first year, and a significant farmlet.year interaction with significantly lower emission per LW for RG-WC compared with the other two treatments in the first year but no significant differences in the second year (Supplementary Table S2).

Discussion

From previous literature, AberMagic has shown a greater WSC and CP concentration in the fresh forage when compared with other ryegrass cultivars (Chen et al., 2016; Moscoso et al., 2019; Wang et al., 2020). In the present experiment, the WSC concentration of the standing forage before being cut for silage was numerically higher for RG-WC and MRG compared to PP (Supplementary Table S3). While it might be anticipated that silage produced from the improved cultivar would maintain this trait for higher WSC concentration, this was not observed, with the PP silage having greater CP, DM and WSC concentration than MRG and being similar in terms of the other silage qualitative variables. The ratio of WSC in silage to WSC in the standing forage may be considered as a proxy for the extent of fermentation, and as such, would suggest that the advantage of the higher WSC content of the MRG is lost through more extensive fermentation under typical ensiling conditions as in the present study. The CP concentration in the standing forage was lower for the improved varieties of ryegrass compared to the PP treatment (Supplementary Table S1), contrary to previous observations (Lee et al., 2003), and the difference was maintained in the respective silages. Nitrogen fixation by the clover in a grass-clover sward can result in the CP concentration of subsequent silage produced to be similar to that of fertilised grass swards (e.g. Bertilsson and Murphy, 2003; Lee et al., 2003; Chen et al., 2016). However, this was not observed in the present study, with CP concentration being lower for RG-WC than the two other

Table 2
Performance and emissions of beef cattle fed with silage produced in farmlets with different types of forage, effects of farmlet, year and the interaction farmlet.year.

Response	Treatment			Average		*P*-value		
	RG-WC	PP	MRG	Year 1	Year 2	Farmlet	Year	Farmlet.Year
ILW (kg)	317	313	315	300[b]	330[a]	0.927	0.003	0.964
FLW (kg)	430	431	424	402[b]	454[a]	0.739	<0.001	0.677
ADG (g/day)	0.71[b]	0.74[a]	0.67[b]	0.65[b]	0.77[a]	0.041	<0.001	0.059
CO_2 (g/day)	5 618	5 532	5 720	5 734[a]	5 513[b]	0.151	0.047	<0.001
CH_4 (g/day)	171	170	174	172	171	0.326	0.847	0.011
CH_4/ADG (g/kg)[1]	5.51 (248)[b]	5.46 (235)[b]	5.59 (270)[a]	5.60[a]	5.44[b]	<0.001	<0.001	0.007
CH_4/LW (g/kg)	0.46	0.46	0.48	0.49[a]	0.44[b]	0.115	<0.001	0.007

Abbreviations: RG-WC = Mixed sward; PP = Permanent pasture; MRG = Reseeded monoculture ryegrass; ILW = Initial liveweight; FLW = Final liveweight, ADG = Average daily gain; LW = liveweight.
[1] Log_e numbers in brackets are the back-transformed means.
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

treatments, suggesting that the quantity of nitrogen fixed in the RG-WC sward did not compensate for the lack of fertiliser nitrogen.

However, the RG-WC silage did have a greater WSC and ME concentration, and a lower fibre concentration, which was not observed by Bertilsson and Murphy (2003) when comparing ryegrass with ryegrass-white clover (50:50) silage. Lee et al. (2003) also identified differences in these indicators when comparing ryegrass and ryegrass-white clover (60:40) silage, and despite also having observed lower ADF and NDF, their grass-legume silage had lower WSC and GE than ryegrass alone.

There is a lack of literature data comparing the chemical composition of silage produced from different ryegrass cultivars, with or without white clover; however, there are studies comparing these forages fresh. Forage yield and quality will be influenced through the growing season by factors including management practices, climatic conditions, botanical composition, soil nutrient availability, and the interaction between these (Moore et al., 2020; Perotti et al., 2021). On the NWFP, differences in the amount and timing of rainfall (Supplementary Fig. S1) between the two study years (43% less rainfall in the second year preceding the first cut silage) influenced both the quantity and quality of the silage produced. According to Harrison et al. (2003), the legume component of a ryegrass-white clover mix had substantially lower concentrations of NDF and ADF than the grass component, and grass-legume mixtures often have an increased CP and decreased fibre concentration when compared with grass alone. This was partially observed in the present study, with the RG-WC silage having a lower fibre concentration, but it also had a lower CP concentration. Delevatti et al. (2019) reported that the application of N fertilizers results in a greater forage yield and CP concentration, as might be expected, which suggests that the N fixed by the white clover in the RG-WC treatment was insufficient to compensate for the lack of N fertilizer applied to that treatment compared with the other treatments (receiving up to 200 kg/ha N) in terms of subsequent silage CP concentration. Soil N level, climate and stage of growth also influence non-structural carbohydrates. Perennial grasses generally have ample non-structural carbohydrate concentration to support silage fermentation (1–2%), and low buffering capacity, which are both desirable for optimum preservation by ensiling. Cussen et al. (1995) reported lower soluble carbohydrate concentration from silage made with ryegrass-clover mixtures compared with just ryegrass, in contrast to the results of the present study and to the observations of Bertilsson and Murphy (2003). During the silage process making, once the grass is mown and wilted, carbohydrates and water will disappear because of plant respiration (that is not interrupted), the growth of undesirable aerobic microorganisms and the addition of bacterial inoculant to reduce the pH (Elferink et al., 2000). The reduction of both is important to control the population of aerobic microorganisms and to increase the sugar content relative to the DM through the evaporation of water (Elferink et al., 2000). A minimum DM content of 30% is recommended (Charmley, 2001) when cutting grass for silage; below that, more carbohydrate is consumed during the fermentation process to lower the pH. With a lower DM silage, more acid will be needed in the solution to lower the pH. This may explain the observed WSC contents in the present study since the DM content of the MRG silage was below 30%.

With the use of selected cultivars of ryegrass and the grass-clover mix seeking to enhance nutritional concentration, differences in animal performance and emissions were expected relative to the PP treatment. However, this was not confirmed, as no differences in CH_4 emissions (per animal) were observed between treatments, and animal performance was better for the PP treatment. There is a lack of published data regarding the performance and CH_4 emissions of beef cattle fed with differing silages, but studies with dairy cows suggest that performance may be enhanced through the inclusion of legumes. Hoffman et al. (1998) reported higher milk production for lactating dairy cows consuming alfalfa (*Medicago sativa*) silage compared with perennial ryegrass silage, and Bertilsson and Murphy (2003) observed greater milk production for dairy cows offered ryegrass-white clover (50:50) silage compared with pure ryegrass silage. Dewhurst et al. (2003), however, observed no significant differences in milk yield for cows offered ryegrass silage, or silage from ryegrass with white or red clover (*Trifolium pratense*) (50:50).

Bica et al. (2022) compared rumen metabolite concentration and CH_4 emissions from beef cattle offered red clover silage or ryegrass silage, but not a mixed sward and observed no significant differences for CH_4 emissions. Wang et al. (2020) conducted an *in vitro* experiment comparing different ryegrass cultivars and concluded that AberMagic resulted in a slightly lower CH_4 yield. Although the fresh AberMagic ryegrass variety has advantages from a nutritional point of view (Wang et al., 2020), these characteristics were not maintained after the ensiling process as observed in the present study, and hence, the anticipated differences in CH_4 emissions and animal performance were not observed.

The enteric CH_4 emissions observed in the present experiment were greater than those suggested by the IPCC tier 1 methodology for western Europe (142.5 g/day) but less than those obtained using the country-specific approach adopted in the UK national inventory since 2018, calculated at 197.7 g/day (Wilkes, 2019). According to Hammond et al. (2009), the chemical composition of the forage may explain about 20% of the variation in the CH_4 yield of cattle on perennial ryegrass pastures, suggesting that there is a limited scope for improvements via modification of the composition of forage species to mitigate CH_4 emissions. However, the inclusion of selected species in pasture may be beneficial, since changes in the efficiency of feed conversion by ruminants can improve livestock production and, hence, CH_4 emissions per unit of animal production, i.e., reducing the emission intensity (Waghorn and Woodward, 2006). This was not observed in the present study, since animal productivity was better for animals offered the PP silage, compared with the MRG and RG-WC silages. Even so, under a more holistic assessment, as highlighted by McAuliffe et al. (2018) for example, systems such as the GR-WC can have a lower C footprint since the emission intensity of the system as a whole is reduced by the low use of inorganic nitrogen fertilizers.

From the present study, there was little or no evidence that silages produced from contrasting forages differed sufficiently in their chemical composition to produce differences in enteric CH_4 emissions from growing beef cattle. There were differences in animal live weight gain, resulting in differences in emission intensity. However, the silage from the 'improved' forage treatments (i.e. high-sugar cultivar for perennial ryegrass and the inclusion of white clover) resulted in greater enteric CH_4 emission intensity, contrary to expectations, possibly because hypothetical nutritional differences in the fresh forage were not maintained through the ensiling process. The results of this study provide a basis for a better understanding of the influence of silage composition and quality on beef cattle CH_4 emissions and performance, but further studies in this area are needed to assess forages with greater differences.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100726.

Ethics approval

This study was approved by the Ethical Review Committee of Rothamsted Research (North Wyke, United Kingdom) under Project Licence number P592D2677 and was conducted according to the Animal Scientific Procedures Act (1986).

Data and model availability statement

The data used in the analyses are available on the North Wyke Farm Platform Data Portal which can be accessed through the link: https://resources.rothamsted.ac.uk/farmplatform.

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Declaration of interest

None.

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