

Acta Agriculturae Scandinavica, Section A — Animal Science



the official journal of the North Association of Agricultural Science



ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/saga20

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To cite this article: Quentin Lardy, Mohammad Ramin, Vibeke Lind, Grete Jørgensen, Mats Höglind, Emma Ternman & Mårten Hetta (2024) Effects of daytime or night-time grazing on animal performance, diurnal behaviour and enteric methane emissions from dairy cows at high latitudes, Acta Agriculturae Scandinavica, Section A — Animal Science, 73:1-2, 28-42, DOI: 10.1080/09064702.2023.2249907

To link to this article: https://doi.org/10.1080/09064702.2023.2249907

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Effects of daytime or night-time grazing on animal performance, diurnal behaviour and enteric methane emissions from dairy cows at high latitudes

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ABSTRACT

This study compared animal performance and enteric methane (CH₄) emissions from dairy cows in a part-time grazing (PTG) system in northern Sweden. Twenty-four Nordic Red dairy cows were allocated to one of two treatments: DAY (10 h daytime pasture access) or NIGHT (12 h nighttime pasture access). The cows in each treatment received the same ad libitum partial mixed ration (PMR) indoors and ad libitum herbage allowance. Methane was recorded using two linked GreenFeedTM emissions monitoring (GEM) units, on pasture and indoors. Day or night grazing showed no statistical differences in estimated grass or PMR intake, milk production or daily enteric CH₄ emissions. There was a rapid decrease in diurnal CH₄ emissions (28%) when the cows were moved from indoors to pasture in both grazing treatments. Using two GEM units (indoor, outdoor) in combination improved the diurnal assessment of enteric CH₄ emissions during PTG conditions in the mixed feeding system.

ARTICLE HISTORY

Received 30 May 2023 Accepted 15 August 2023

KEYWORDS

Part-time grazing; access time: mixed ration: behaviour; pasture; intake; diurnal pattern

Introduction

Dairy production in the Scandinavian countries is characterised by relatively high-yielding cows with continuous calving, in combination with a short grazing season lasting 2-4 months (Kismul et al., 2019). The dairy system is mainly based on indoor feeding (silage and concentrate) throughout the year, combined with part-time grazing (PTG) during the summer season. Grazing can be beneficial from an animal welfare point of view and also lowers feed costs (Finneran et al., 2012; Wright, 2019) and reduces enteric methane (CH₄) production (Cameron et al., 2018). Keeping cows full-time on pasture can be challenging (Wilkinson et al., 2020), but by using a PTG system farmers can meet consumer and societal demand for sustainable, pasture-based dairy farming (Krizsan et al., 2021) while maintaining an adequate production level.

Farmers can customise various aspects of their PTG strategy to optimise production by adapting to local conditions. Some previous studies comparing PTG strategies with indoor feeding and full-time grazing have found that dry matter intake (DMI) and milk production are not affected by PTG (Vibart et al., 2008; Mendoza et al., 2016), while others report a reduction in milk production and DMI on PTG compared with permanent indoor housing (Soriano et al., 2001; Bargo et al., 2002; O' Neil et al., 2011; Civiero et al., 2021).

Feed intake is the main driver of milk yield and enteric CH₄ production from dairy cows (Ramin & Huhtanen, 2013), but in any system involving grazing cows, including PTG systems, it is difficult to measure DMI on pasture. Some studies have shown that grazing can reduce total CH₄ production (g d⁻¹) or CH₄ intensity (g kg milk⁻¹) compared with indoor feeding, and that the reduction in CH₄ is greater than the decline in milk yield (O'Neil et al., 2011; Mufungwe et al., 2014; Civiero et al., 2021). Other studies have found no effects of grazing on milk production or CH₄ emissions from grazing cows (Dall-Orsoletta et al., 2016; Cameron et al., 2018). Enteric CH₄ emissions from grazing dairy cows can be recorded using several techniques such as the sulphur hexafluoride (SF₆) tracer technique (Pinares-Patiño & Clark, 2008) or direct measurements of emissions during milking by the sniffer technic (Garnsworthy et al., 2012). However, neither of these techniques can monitor short-term effects on CH₄ emissions over the course of a day.

In PTG systems with both indoor feeding and grazing, it can be complicated to record CH₄ emissions over an extended period as the cows move between pasture and barn daily. As a result, the effects of each feeding environment on CH₄ emissions in a mixed feeding system are not fully known and a more accurate measuring approach is needed to map this relationship. The GreenFeedTM emissions monitoring (GEM) system (C-Lock Inc., Rapid City, SD) can record CH₄ emissions indoors (Huhtanen et al., 2015) and under grazing conditions (Waghorn et al., 2016) over unlimited periods. Several GEM units placed in different feeding environments allow the potential short-term effects of these environments on CH₄ emissions to be recorded. One GEM located on the pasture, and one installed in the barn enable the evaluation of PTG strategies to mitigate CH₄ emission, but use of indoor and outdoor GEM units in a mixed feeding system has not been published previously.

Ruminants on full-time pasture show diurnal grazing and resting behaviours related to photoperiod, with dusk grazing being the longest and most intense of all grazing events during the day (e.g. Orr et al., 1997; Gibb et al., 1998; Taweel et al., 2004). The long day photoperiod with prolonged twilight in summer in Northern Scandinavia, combined with higher herbage feeding value (Delagarde et al., 2000), may encourage cows to graze for longer in the evening. For example, Sairanen et al. (2006) found a trend for increased herbage intake and milk yield during night grazing in a PTG experiment in Finland, while Orr et al. (2001), Abrahanse et al. (2009) and Vibart et al. (2017) all observed increased milk yield, as well as increased fat and protein yield during evening grazing. However, there is little information available about PTG management under long-day conditions.

To fill the above-mentioned research gaps, this study compared the effects of daytime and night-time grazing on animal performance, diurnal behaviour and enteric CH₄ emissions from dairy cows in a PTG system at high latitudes. The CH₄ emissions were measured on pasture and indoors, using two GEM units.

Materials and methods

The grazing experiment was carried out from 1 June to 2 July 2021 at the Röbäcksdalen research farm, Swedish University of Agricultural Sciences (SLU), Umeå, Northern Sweden. The farm (63.81°N 20.23°E) is part of the Swedish Infrastructure for Ecosystem Science (SITES). Average temperature during the experimental period was 17 °C, which was somewhat above the 30-year average at the nearest weather station (12.8 °C) (SMHI, 2020). There was no rainfall during the study period, compared with a 30-year average for June of 48.7 mm (SMHI, 2020). The sun rose at 02:30 h and set at 22:30 h, but at this time of the year there is civil twilight at high latitudes, i.e. there is no true darkness during the night. All use of animals in the study and the experimental protocol were approved by the Swedish Ethics Committee on Animal Research (Permit A 6-2021), represented by the Court of Appeal for Northern Norrland in Umeå, in line with Swedish laws and regulations implementing EU Directive 2010/63/EU on animal research.

Experimental design and routine

Animals and treatments

A total of 30 Nordic Red dairy cows were used in the study. The animals were blocked according to days in milk (DIM), milk yield (MY) and parity (primiparous and multiparous) and allocated to one of two grazing treatments: daytime grazing (DAY) or night-time grazing (NIGHT). The DAY group was kept on pasture for 10 h during the day (07:00-17:00 h) and the NIGHT group for 12 h during the evening and night (17:00-05:00 h). The DAY and NIGHT had, respectively, on average (SD) DIM 185.9 (27.5) and 198.3 (27.5), parity 1.6 (0.22) and 1.8 (0.29), and MY 28 (1.7) and 28 (1.6) kg per groups. All animals in both treatments received an ad libitum partial mixed ration (PMR) indoors and an ad libitum herbage allowance from daily fresh strips. Following the milking schedule on the research farm, the cows were milked twice daily, around 06:00 and 17:00 h for the DAY group, and around 16:00 and 05:00 h for the NIGHT group. Movement of the animals between barn and pasture occurred after milking. When not on pasture, the cows were kept indoors in a loose-house dairy barn. The experiment lasted 31 days, with a 24day (1-25 June) period of adaptation to feed, management routines and visiting the GEM units, followed by seven days of recording (26 June-2 July).

Grazing and pasture allocation

Two adjacent paddocks (each 2.6-3 ha) of cultivated grass-clover ley, sown two and three years previously, respectively, were used for grazing. The botanical composition of the leys, estimated using the dry-weight rank method of Mannetje and Haydock (1963), was: 37% timothy (Phleum pratense), 29% white clover (Trifolium repens), 21% meadow fescue (Festuca pratensis) and 13% other species.

The pasture was divided into two daily consecutive strips, one for the DAY group (offered after morning milking) and one for the NIGHT group (offered after afternoon milking). Strip grazing was employed and an estimated herbage allowance on pasture of 18 kg dry

matter (DM) cow⁻¹ d⁻¹ was provided in both treatments, which was three times the expected pasture intake (6 kg DM $cow^{-1} d^{-1}$) to ensure ad libitum herbage allowance. The animals on pasture had access to a GEM unit, fresh water, and a salt block in each strip. While the animals were being milked, a new strip was set up, using front and back electric fences, and all equipment was moved to the new strip.

To determine the required strip area, pre-grazing herbage mass was estimated daily by walking the paddock in a 'W' shape and measuring compressed sward heights at 50 points using a modified rising plate metre (Mould, 1992). Herbage availability (kg DM cow⁻¹ d⁻¹) was estimated based on a linear regression relationship between compressed sward height (cm) and herbage mass (kg). The regression model used to determine herbage availability was calibrated three times during the recording period (day 0, day 3, day 5) by measuring sward height 20 times with the plate metre and then immediately cutting squares of 0.16 m² to approximately 3 cm with an electric clipper (Bosch Iso cordless grass shears, Robert Bosch GmbH, Germany). The harvested biomass was dried at 60 °C for 72 h. Post-grazing herbage mass, measured daily with the same method, was used as an index of ad libitum herbage allowance.

Housing and indoor feeding

Indoors, the animals had access to 15 feed bunks (Roughage Intake ControlTM, RIC, Insentec B. V., Marknesse, The Netherlands), one GEM unit, one concentrate feeding station (SAC, S.A. Christensen and Co. Ltd., Kolding, Denmark) and one self-filling water trough. The cows were fed the PMR ad libitum, with

Table 1. Chemical composition and nutritive value of the partial mixed ration (PMR), silage, base concentrate and protein concentrate (tabulated values from manufacturer) fed to the dairy cows in this study.

	PMR ^a	Silage ^b	Base concentrate ^c	Protein concentrate ^d
DM (g kg ⁻¹)	450	305	880	890
ME (MJ kg DM ⁻¹)	11.8	10.4	13.4	13.4
CP (g kg DM ⁻¹)	186	148	180	350
NDF (g kg DM ⁻¹)	351	513	225	270
OM (g kg DM ⁻¹)	-	923	_	-

Abbreviations: PMR, Partial mixed ration; DM, dry matter; ME, metabolisable energy; CP, crude protein; NDF, neutral detergent fibre; OM, organic matter.

fresh feed delivered twice daily in each treatment (one delivery immediately after milking). A stationary feed mixer (Nolan A/S, Viborg, Denmark) was used to process the PMR, which consisted of (DM basis): 500 g kg⁻¹ silage, 490 g kg⁻¹ concentrate with 440 g kg⁻¹ of base concentrate (Komplett Norm 180, Lantmännen Lantbruk, Malmö, Sweden), 50 g kg⁻¹ of protein concentrate (Addera Bas 350, Lantmännen Lantbruk, Malmö, Sweden) and 10 g kg⁻¹ minerals. Concentrates were fed in the PMR, the GEM units and the concentrate feeder in the barn. Base concentrate was used forthe concentrate feeder (daily max of 0.5 kg feed per cow) and the GEM unit (daily max of 2 kg feed per cow). The grass silage was from the first cut (2020) of mixed leys of timothy, meadow fescue, and red clover. The chemical composition of the feeds is shown in Table 1.

Experimental measurements

Feed quality and composition

The chemical composition of the silage was analysed using near-infrared spectrophotometry (NIRS) by Eurofins (Agro Testing Sweden AB, Kristianstad, Sweden), according to the research farm's routines. Information on the composition of the concentrates was provided by the manufacturer (Lantmännen Lantbruk AB, Malmö, Sweden). Herbage samples (n = 30)for analysis of chemical composition were hand-picked daily, mimicking the herbage strata grazed, before the animals entered the pasture, by walking in the paddock as described by Smit et al. (2005). The herbage samples were pooled, dried at 60 °C for 72 h and milled into 1 mm particles before being sent to NIBIO (Særheim, Norway) for chemical analysis by NIRS as described by Fystro and Lunnan (2006). Metabolisable energy (ME) content of the herbage was calculated according to Lindgren (1979), based on in vitro organic matter digestibility (IVOS) determined at the SLU laboratory (Uppsala, Sweden).

Animal performance

Feed intake of PMR was recorded automatically at the feed bunks, which recorded fresh feed intake (kg) on each individual visit by each animal. Intake (kg DM day⁻¹) was determined by accounting for the DM content of each ingredient in relation to its proportion in the overall diet formula. Individual concentrate intake (kg) was also recorded automatically at the two GEM units and the concentrate feeder.

To estimate total DM intake (TDMI) in lactating cows, the equation developed by Souza et al. (2019) and presented in NASCEM (2021) was used, with adjustment for calculating milk energy and a fixed value for body

^aCalculated value based on proportion and feed value of each ingredient.

^bAnalysed by Eurofins (Food and Agri Sweden AB, Lidköping, Sweden).

^cKomplett Norm 180, tabulated values from manufacturer (Lantmännen Lantbruk AB, Malmö, Sweden).

^dAddera Bas 350, tabulated values from the manufacturer (Lantmännen Lantbruk AB, Malmö, Sweden).

(3)



condition. Values were averaged per animal for the recording week. The equation took the form:

TDMI =
$$[(3.7 + Parity \times 5.7) + 0.305$$

× MilkE (Mcal d⁻¹) + 0.022 × BW (kg)
+ (-0.689 + Parity × -1.87) × BSC]
× [1-(0.212 + Parity × 0.136) × e(-0.053 × DIM)] (1)

where TDMI is total dry matter intake (kg d^{-1}), parity is 0 for primiparous and 1 for multiparous, MilkE (milk energy) was calculated as energy-corrected milk yield multiplied by 3.14 according to Sjaunja et al. (1990) and then converted into Mcal by divided by 4.184, BW is body weight of the animal (kg), BCS is body condition score (set by default for all animals at 3.5) and DIM is number of days in milk at the beginning of the recording period.

To ensure that the estimated TDMI and herbage intake were consistent, the animal's energy requirements were compared with dietary energy supply and the animal's intake capacity. The Nordic feed evaluation system (NorFor, 2011; NorFor Feedstuff Table revision 2.10 and NorFor Feed Ration Calculator revision 2.15) were used for estimating the dietary fill value. The energy requirements across all animals were on average fulfilled to 101% (± 8.3), and the intake capacity to 94% (\pm 5.1). Herbage dry matter intake (kg DM d⁻¹) was estimated by subtracting the recorded intakes (PMR intake and concentrate intake) from the TDMI.

Morning and afternoon milk yield was recorded with gravimetric milk recorders (S.A. Christensen & CO, Kolding, Denmark) for all animals during the recording period. Milk subsamples were collected at morning and afternoon milkings during the last 48 h of the experiment. The samples were pooled separately for morning and afternoon in plastic bottles, preserved with 2bromo-2-nitropropane-1,3-diol (Bronopol, Valio Ltd., Helsinki, Finland), stored at 4 °C and sent for analysis of fat, protein and lactose content by mid-infrared spectroscopy (Combiscope 600 HP, Delta Instruments, Drachten, The Netherlands) at the SLU laboratory (Uppsala, Sweden). The ECM values (kg d⁻¹) were calculated based on milk composition data according to the equation of Sjaunja et al. (1990):

where MY is milk yield (kg d⁻¹) and fat, protein and lactose content is the mean value of four consecutive milkings per cow ($q d^{-1}$)

Methane recordings

Methane emissions were measured using two GEM units, one mobile unit located out on pasture as described by Waghorn et al. (2016) and one stationary unit in the barn as described by Huhtanen et al. (2015). These two GEM units were linked wirelessly and considered as one unit in calculation of CH₄ emissions of the individual animals. The indoor unit was installed in a corner of the barn and insulated by a wooden panel to avoid eruptive interference from nearby animals. This unit was calibrated weekly with a span gas for calibration (mixture of CO_2 , CH_4 and O_2) and zero gas (N_2) . The outdoor unit was mounted on a trailer, powered by solar panels, and equipped with a wind sensor designed by the manufacturer (C-Lock Inc., Rapid City, SD, USA). Animal access to the pasture GEM unit was managed by a chute, to reduce disturbance from other animals while visiting the unit. The pasture GEM unit was calibrated automatically with the same span gas and zero gas as the indoor unit. A CO₂ recovery test was conducted prior to the recording period for both GEM units. Airflow rates and gas concentrations were measured continuously and volumetric flux (L min⁻¹) of gases emitted by the animals was calculated. Head position of the animal was recorded by the system during each visit, and recordings with inappropriate head positions were filtered out by the system. The experimental settings were identical for both GEM units and allowed cows to visit a unit at minimum 4-h intervals. During each visit, the cows were given a maximum of eight drops of 50 g of base concentrate. Daily CH_{Δ} emissions (g d⁻¹) were calculated as:

$$\label{eq:CH4Combined GEM} CH_4 Combined GEM = [CH_4 Outdoor GEM(g d^{-1}) \\ \times \mbox{ visits Outdoor GEM} + CH_4 Indoor GEM(g d^{-1}) \\ \times \mbox{ visits Indoor GEM]/(visits Indoor GEM+} \\ \mbox{ visits Outdoor GEM)}$$

where CH₄ Combined/Outdoor/Indoor GEM is in g CH₄ cow⁻¹ d⁻¹ and visits is number of validated visits to the GEM units (indoor and outdoor).

The CH₄ emissions for the combined GEM unit were calculated by averaging the values obtained over the recording period. To be considered valid in the experimental design, the combined GEM values for CH₄ emissions had to have at least one recording from each unit per day, to ensure a balance (indoor, outdoor) in values. If this criterion was not met, the observation was reported as missing data. Using this data management approach, 73% of daily CH₄ observations for the combined GEM were considered valid. When investigating the differences recorded between the GEM units and

their feeding environment, all available valid data (CH₄ and visits) from the two GEM units were used.

Figures (panel A) show the diurnal pattern of enteric CH₄ emissions plotted using arithmetic mean hourly CH₄ emissions values ($a h^{-1}$, with error bar) for DAY (Figure 1) and NIGHT (Figure 2). All validated visits to each GEM unit per hour during the recording period were used (n = 292 for DAY, n = 314 for NIGHT). Hourly CH₄ emissions values and visits for each indoor and outdoor GEM unit per treatment were computed as arithmetic mean of all recorded measurements per GEM unit, with standard error.

Animal behaviour

All animals were equipped with Nedap SmartTag Neck sensors (NT; Nedap Livestock Management, DC Groenlo, The Netherlands), which automatically recorded four different behavioural states (eating/ grazing, ruminating, resting, other). The SmartTag sensors have been validated for use in measuring indoor (Borchers et al., 2021) and outdoor (Rue et al., 2020) behaviour. Behaviour information was obtained as datasets of observations for each cow at 1-min intervals, which were summarised per day prior to statistical analysis. In addition, the datasets were split according to cow location (indoor or on pasture). Any outliers identified defined as 1.5 times the interquartile range (IQR = Q3 - Q1) greater than the third quartile (Q3), or 1.5 times the interquartile range less than the first quartile (Q1) were removed from the dataset for the particular experimental day. As outdoor access duration differed between the treatments, grazing behaviour were expressed as grazing duration (h), but also as grazing time as a percentage of access time. The diurnal pattern of eating (indoor) and grazing (outdoor) behaviour in the figures (panel B) were computed using arithmetic hourly eating/grazing behaviour means per treatment over the recording period.

Statistical analysis

Cows with low incidence or lack of voluntary visits to the GEM unit were removed from the analysis. Certain animals were avoiding the outdoor GEM unit in particular. The threshold of voluntary visits was set to 3.5 visits per GEM units (n = 2) over the recording period of 7 days. The animals under this threshold were removed from the statistical analysis.

Statistical analysis was performed, and diagrams were prepared using R software (R Core Team., 2021). The animal variables were averaged per cow (n = 24) over the recording period resulting in animal period (mean of 7 days per cow) as the experimental unit. All data on feed intake (measured and estimated), milk, CH₄, GEM visits and behaviour were subjected to ANOVA using a GLM procedure to test for effects of the two grazing treatments (DAY, NIGHT), with DIM, parity and pre-experimental MY as covariates. These co-variates were excluded when analysing CH₄ emissions and cow behaviour, because they did not improve the model. Least square means were calculated using the LSMEANS package in R and significant pairwise differences between treatments were determined using Tukey-Kramer adjustment ($p \le 0.05$).In the present study, the effect size (Cohen's d) was used to quantify the difference between the 'DAY' and 'NIGHT' treatment group. Effect sizes were reported in the results when d >0.8, indicating moderate to large effects.

A second statistical model was employed to compare indoor and outdoor CH₄ emissions recorded. The CH₄ data were daily mean emissions per animal and GEM unit resulting in predicted daily CH₄ emission per cow and unit as the experimental unit. A mixed-effects model was used, with the individual cow considered as random factor and GEM units environment (indoor or pasture) as fixed factor. The LSMEANS were calculated using the LSMEANS/PDIFF option in R and significant pairwise differences between treatments were determined using Tukey-Kramer adjustment ($p \le 0.05$).

Results

Six cows (five in the DAY group, one in the NIGHT group) were removed from the analysis in the study due to very low incidence or lack of voluntary visits to the GEM unit on pasture. After removing these animals, the groups were composed of 10 cows in DAY and 14 cows in NIGHT and had, respectively: average (SD) live weight (LW) 657 (73.6) and 593 (70.8) kg, DIM 216 (101) and 240 (124), parity 1.6 (0.80) and 1.8 (0.84), and MY 27 (2.2) and 28 (1.5) kg.

Feed and pasture quality

The herbage had 42% lower DM content than the PMR, a similar energy content (11.3 and 11.8 MJ ME kg DM⁻¹, respectively) and crude protein content (186 g kg DM⁻¹), and a higher neutral detergent fibre (NDF) content than the PMR (Table 1). The chemical composition of the herbage samples (Table 2) collected from each treatment strip was found to be numerically similar.

Pasture characteristics and chemical composition of the herbage were similar in the two treatments (Table 2, no statistical testing). Pre- and post-grazing compressed height was identical in the two treatments. However, there was high standard deviation in daily

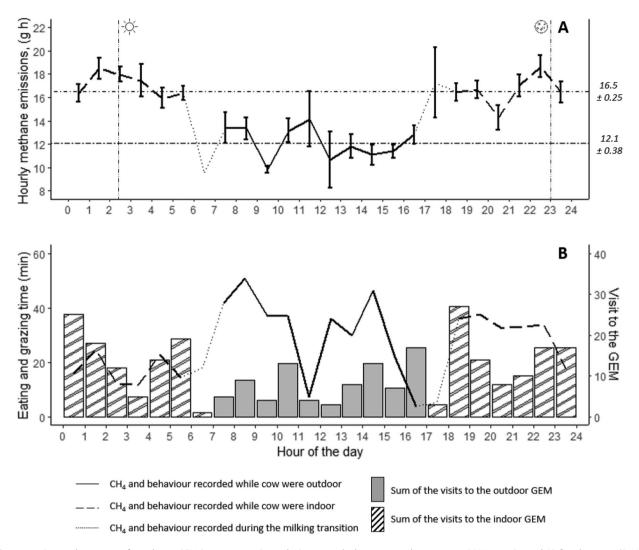


Figure 1. Diurnal pattern of methane (CH₄) emissions (panel A), eating behaviour and visits to a GEM unit (panel B) for daytime (DAY) pasture access (07:00–17:00 h). (A) Mean enteric CH₄ emissions (g h⁻¹) per hour (7-day means of all validated CH₄ recordings, n = 292 for DAY), where horizontal lines indicate mean CH₄ recorded by each unit (upper line = indoor GEM, lower line = outdoor GEM), and the vertical dashed line at 02:30 h represents sunrise and the vertical dashed line at 23:00 h represents sunset. (B) Mean eating time (min) per hour recorded with the Nedap system, where bars represent sum of visits per hour to the accessible GEM unit at that time of day (filled for indoor, cross-hatched for outdoor). The lines (CH₄ and eating behaviour) in panels A and B are identical, showing the location of the animals at a given time of day (dotted line while cows were indoors, dotted line transition during milking, solid line while cows were outdoors).

strip area and pre-grazing herbage mass, due to winter damage to a section of the sward that had suffered from inundation, resulting in limited grass growth in one strip per treatment. Grazing strip area was increased in those cases to ensure sufficient herbage availability.

Animal performance

Intake of the PMR (kg DM d⁻¹) did not differ statistically between the treatments (p = 0.317), but intake of concentrate in the GEM units and the concentrate feeder was significantly higher for DAY treatment than the NIGHT grazing (p = 0.006) (Table 3). Estimated herbage

DMI was similar in the two treatments (p = 0.575) (Table 3). Estimated TDMI differed significantly (p = 0.012), with cows in the DAY treatment consuming more feed (21.7 kg DM) than those in the NIGHT treatment (20.2 kg DM) (p = 0.012). Total forage intake (silage + herbage) did not differ statistically significantly between the treatments (12.5 and 11.8 kg DM d⁻¹ for DAY and NIGHT cows, respectively; p = 0.168), but there was a tendency for a difference in total concentrate intake (9.0 and 8.3 kg DM d⁻¹ for DAY and NIGHT, respectively; p = 0.069). The effect size (d) of most intake variables (PMR, estimated herbage DMI, concentrate intake and total forage intake) were

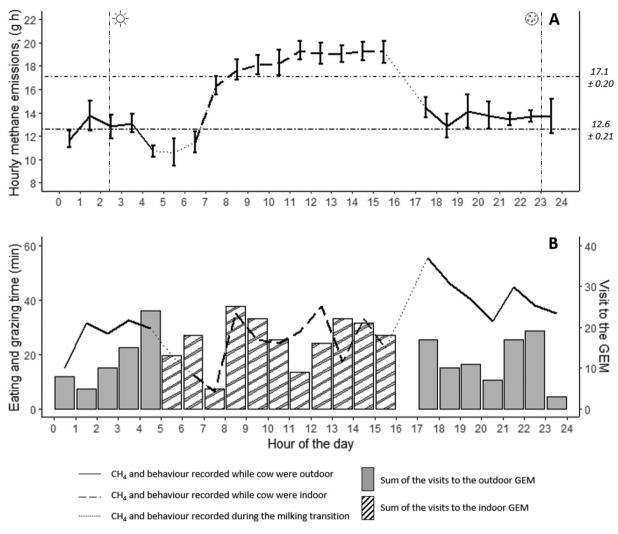


Figure 2. Diurnal pattern of methane (CH₄) emissions (panel A), eating behaviour and visits to a GEM units (panel B) for night-time (NIGHT) pasture access (17:00–05:00 h). (A) Mean enteric CH₄ emissions (g h⁻¹) per hour (7-day means of all validated CH4 recordings, n = 314 for NIGHT), where horizontal lines indicate mean CH₄ recorded by each unit (upper line = indoor GEM, lower line = outdoor GEM), and the vertical dashed line at 02:30 h represents sunrise and the vertical dashed line at 23:00 h represents sunset. (B) Mean eating time per hour (min) recorded with the Nedap system, where bars represent sum of visits per hour to the accessible GEM unit at that time of day (filled for indoor, cross-hatched for outdoor). The lines (CH₄ and eating behaviour) in panels A and B are identical, showing the location of the animals at a given time of day (dotted line while cows were indoors, dotted line transition during milking, solid line while cows were outdoors).

superior to 0.8, indicating a potential difference in favour to the DAY treatment which was not detected by our statistical model but resulted in a statistically significant difference for TDMI. Milk yield (expressed as ECM) and milk composition did not statistically differ between the two treatments (p > 0.05) (Table 3).

Enteric methane emissions and GEM unit visits

There was no statistically significant effect of the treatments on absolute CH_4 emissions (g cow^{-1} d⁻¹) from the combined indoor and outdoor GEM units (p > 0.05; d < 0.2). Moreover, CH_4 intensity (g CH_4 kg ECM^{-1}) and CH_4 yield (g kg DMI^{-1}) did not differ significantly (p >

0.05) between DAY and NIGHT treatments (Table 4). The results from the second statistical model, comparing indoor and outdoor emissions, revealed a statistically significant difference (p < 0.0001), with the average CH₄ value recorded in the outdoor GEM unit being lower (300 g CH₄ cow⁻¹ d⁻¹ or 12.5 g CH₄ cow⁻¹ h⁻¹) than that recorded in the indoor GEM unit (414 g CH₄ cow⁻¹ d⁻¹ or 17.2 g CH₄ cow⁻¹ h⁻¹).

The indoor (p < 0.0001) and outdoor (p = 0.006) GEM visit frequencies differed significantly between the treatments, whereas combined visits to the GEM units showed no statistically differences (p = 0.116; d > 0.8). Cows in the DAY and NIGHT treatments visited the GEM units 3.9 and 3.3 times per day, respectively, with

Table 2. Average sward characteristics and composition of the herbage offered in the DAY (daytime) and NIGHT (night-time) treatments (± SD).

	Treatment		
	DAY	NIGHT	
Sward characteristics $(N = 5)$			
Daily strip area (m ²)	3758 ± 1057	3854 ± 998	
Pre-grazing herbage height ^a (cm)	20 ± 0.5	20 ± 0.5	
Pre-grazing herbage mass (kg DM ha ⁻¹)	2684 ± 524	2735 ± 556	
Post-grazing herbage height ^a (cm)	13 ± 1.5	13 ± 1.2	
Herbage availability (kg DM cow ⁻¹)	16 ± 0.9	17 ± 1.3	
Herbage chemical composition ($N = 10$)			
ME ^b (MJ kg DM ⁻¹)	11.3 ± 0.73	11.3 ± 0.78	
Digestibility ³ (g kg DM ⁻¹)	742 ± 36.6	749 ± 38.2	
CP ^c (g kg DM ⁻¹)	172 ± 7.0	172 ± 10.4	
NDF ^c (g kg DM ⁻¹)	483 ± 32.8	479 ± 30.7	
OM ^c (g kg DM ⁻¹)	908 ± 10.1	906 ± 6.1	

Abbreviations: DM, dry matter; ME, metabolisable energy; CP, crude protein; NDF, neutral detergent fibre; OM, organic matter: SD, standard deviation. ^aMean of 50 measurements taken with a rising plate metre (compressed height) per day per treatment.

^bIn vitro VOS (organic matter digestibility) method performed at the SLU Uppsala laboratory, metabolisable energy calculated according to Lindgren (1979)

^cNear-infrared spectrometry performed at the NIBIO Sarheim laboratory.

an average of 27 and 23 visits per cow, respectively, over the entire recording period. The distribution of visits was statistically significantly different between the two treatments, with the DAY treatment making a greater proportion of visits (75%) to the indoor GEM (2.9 visits per cow per day) than the outdoor GEM (0.9 visits per cow per day) and the NIGHT treatment visiting both units equally, indoor unit on 51% of the visits (1.7 daily visits) and outdoor unit on 49% (1.6 daily visits).

The differences in CH₄ emissions between indoor and outdoor measurements are shown in Figure 1(A) (DAY)

Table 3. Performance of Nordic Red dairy cows in the two treatment groups (least square mean) and effect of the daytime grazing (DAY) and night-time grazing (NIGHT) treatments (SEM and p-value).

	Treatment			
	DAY	NIGHT	SEM	<i>p</i> -value
Dry matter intake (DMI; kg DM	d^{-1})			
PMR intake	15.2	14.5	0.67	0.317
Concentrate intake ^a	1.4	1.1	0.09	0.006
Estimated herbage intake ^b	4.9	4.5	0.42	0.575
Estimated TDMI ^c	21.6	20.1	0.52	0.012
Milk				
Milk yield (kg d ^{–1})	26.3	26.0	0.67	0.598
ECM ^d (kg d ⁻¹)	29.0	28.2	1.15	0.490
Milk fat ^e (%)	4.5	4.6	0.21	0.586
Milk protein ^e (%)	3.8	3.7	0.14	0.578
Milk lactose ^e (%)	4.5	4.4	0.08	0.517

Abbreviations: DMI, dry matter intake; PMR, partial mixed ration; TDMI, total dry matter intake; ECM, energy-corrected milk; SEM, standard error of mean.

Table 4. Effects of the daytime (DAY) and night-time (NIGHT) treatments (SEM and p-value) on enteric methane emissions and GEM metrics (least square mean) for the dairy cows in this study.

	Treatment			
	DAY	NIGHT	SEM	<i>p</i> -value
Methane production (g d ⁻¹)				
CH₄ Combined GEMa	373	370	21.1	0.881
CH₄ Indoor GEM	399	426	22.9	0.267
CH ₄ Outdoor GEM	285	301	22.8	0.484
Methane related to performance ^b				
CH ₄ intensity ^c (g kg ECM ⁻¹)	13.4	13.4	1.21	0.997
CH ₄ yield ^d (g kg DMI ⁻¹)	17.3	18.3	0.98	0.280
Visits to the GEM units (visits d ⁻¹) ^e				
Combined GEM ^a	3.9	3.3	0.32	0.116
Indoor GEM	2.9	1.7	0.23	<.0001
Outdoor GEM	0.9	1.6	0.22	0.006

Abbreviations: CH₄, methane; ECM, energy-corrected milk; DMI, dry matter intake: GEM, GreenFeed emissions monitoring unit: SEM, standard error of mean

and Figure 2(A) (NIGHT). As can be seen from these diagrams, there was a shift in emissions during the milking transition, while the hourly means for each treatment and unit was statistically similar between the two treatments (p > 0.05). This shift occurred to a similar proportion (28%) in both treatments (DAY and NIGHT). For the DAY and NIGHT treatments, indoor emissions were 16.5 and 17.1 g cow⁻¹ h⁻¹, respectively, and outdoor emissions were 12.1 and 12.6 g cow⁻¹ h⁻¹, respectively. The effect size (d > 0.8) showed a difference in CH₄ recorded on the indoor GEM unit for the DAY treatment compared to the NIGHT treatment. The distribution of visits per hour to the GEM are shown in Figures 1 and 2(B). The DAY group showed an unbalanced pattern, while the NIGHT group was more balanced in its distribution of visits throughout the day. The cows were found to graze and visit the GEM units in a similar pattern, which was more evident in the NIGHT treatment, especially when on pasture.

Animal behaviour

As can be seen in Figures 1 and 2(B), there was a peak in grazing behaviour by the animals after being released on pasture, when cows in the DAY treatment engaged in grazing activity for 60% of the time and cows in the NIGHT group engaged in grazing activity for 90% of the time. There was a somewhat similar increase in eating behaviour by the animals when they were

^aConcentrate consumed in the concentrate feeder and the GEM units.

^bEstimated based on TDMI (NASCEM, 2021) minus recorded intake indoors.

^cEstimated from the NASCEM equation (2021).

^dEnergy-corrected milk calculated as in Sjaunja et al. (1990).

^eMilk analysis was performed on four consecutive samplings on the last two days of the recording period.

^aCombined GEM unit values are sum of {emissions value multiplied by number of visits per GEM unit), divided by total number of visits.

^bUsing the CH₄ combined GEM for CH₄ intensity and yield.

^cEnergy-corrected milk calculated as in Sjaunja et al. (1990).

dWhere total DMI was estimated according to the NASCEM equation (2021) based on animal information and feed characteristics.

^eAverage value of visits per cow per day on both GEM, indoor GEM, and outdoor GEM.

indoors. Eating activity decreased for all cows during the civil twilight period (22:30-02:30 h).

There was no statistical difference in average duration of eating/grazing (p = 0.274), inactivity (p = 0.804) or rumination over the average 24-h period (p = 0.502) (Table 5). The cows in the NIGHT treatment spent 0.8 h more time grazing (p < 0.001) than those in the DAY treatment. However, time dedicated to grazing as a percentage of access time to pasture was higher for the DAY treatment (p = 0.002) than the NIGHT grazers. The NIGHT cows had a higher grazing activity during the first two hours on pasture compared with DAY cows' grazing activity (p < 0.004).

Discussion

In this short-term study, we showed that CH₄ emissions were reduced at pasture for both DAY and NIGHT groups, indicating the potential of fresh grass inclusion in the diet to reduce CH₄ emissions over a short period in a PTG system, also shown by Koning et al. (2022). However, this study used a simple experimental design and corresponding statistical model, which might have limited the evaluation of the results.

Herbage, feed intake and milk response

Herbage quality is an important parameter in grazing studies, with grass digestibility, often expressed as ME concentration of the herbage, being one of the most commonly used parameters (Waghorn & Clark, 2004). Herbage ME concentration during early summer on farms in northern Sweden has previously been reported to range between 10.1 and 10.9 MJ ME kg DM⁻¹ (Spörndly, 2003). The herbage grazed in the present

Table 5. Effects of pasture access in daytime (DAY) and nighttime (NIGHT) (least square mean) on the behaviour of Nordic dairy cows and their specific grazing behaviour on pasture (SEM and p-value).

	Treatment			
	DAY	NIGHT	SEM	<i>p</i> -value
Animal behaviour (h)				
Eating time	7.4	7.7	0.23	0.274
Ruminating time	7.8	7.6	0.31	0.502
Inactive time	8.1	8.2	0.42	0.804
Grazing behaviour ^a				
Grazing duration (h)	3.9	4.7	0.22	0.001
Grazing duration per access time ^b (%)	38.9	32.7	1.86	0.002
Grazing activity ^c (h)	1.1	1.4	0.08	0.004

Abbreviations: SEM, standard error of mean.

study had a higher ME concentration than this, indicating above-average herbage quality (e.g. Spörndly & Wredle, 2005; Kismul et al., 2019). Silage NDF was higher than the herbage NDF but due to the concentrate inclusion, the PMR ended up with a higher NDF content than the grazed grass. The crude protein concentration in herbage was similar to that reported in the studies cited above (191 and 156 g kg DM⁻¹, respectively) and to that in the PMR, and was considered sufficient for animal performance.

Cows in the DAY and NIGHT treatments had similar PMR intake (approximately 70% of TDMI), despite the difference in time spent indoors. The animals spent 3.5 h (DAY) and 3.0 h (NIGHT) eating indoors to achieve the same recorded indoor intake. Cows in a study by Gomez and Cook (2010) spent on average 4.3 h d⁻¹ eating indoors in a commercial free-stall barn, indicating that the time of access to indoor feed in our study did not limit feed intake.

The DAY treatment achieved higher estimated TDMI than the NIGHT grazers, as a result of the numerically higher PMR intake, herbage intake and concentrate intake by DAY cows which is also shown by their high effect size (1.02, 0.85 and 1.80, respectively). The accuracy of the TDMI estimates, and therefore of the herbage DMI values, was insufficient to allow pertinent conclusions on the impact of the treatments on the intake variables. The magnitude of the effect of all other input variables illustrates the positive difference for the DAY treatment on DMI. This is logical since most consumption takes place indoors (70% for both group), and the magnitude of the differences is high (d = 1.02) for the recorded PMR consumption.

The loss of 5 animals in the DAY group resulted in a numerically higher average body weight, which could have influenced the TDMI estimated from the NASCEM equation. A more complex design might have highlighted statistically this difference between the treatment. A cross-over design would have allowed greater statistical power, as suggested by Huhtanen & Hetta (2012), but this type of design is challenging in grazing trials with lactating dairy cows. Parameters such as differences in photoperiod (day length) and growing conditions (e.g. herbage quality) between periods might cause animal x period interactions causing disturbances in the data analysis (Morris, 1999). This concern is even greater at high latitudes with short and intensive vegetative season.

Another challenge in evaluating grazing experiments is to define the experiential unit, as the recordings of grazing animals are not independent of each other when confined in the same paddock (Fisher, 2000). Another method of improving statistical power of the

^aWhen eating behaviour took place on pasture, it was interpreted as grazing behaviour.

^bPercentage of access time, 10 h for DAY (07:00–17:00 h) and 12 h for NIGHT (17:00-05:00 h).

Grazing activity during the first two hours of pasture access (07:00–09:00 h for DAY cows, 17:00-19:00 h for NIGHT cows).

intakes could be to use daily individual recordings in our model instead of a sampling week average. On the other hand, as it is not possible to estimate daily body weight changes of individual cows in short-term trials (Morris, 1999), and as there would be a strong dependency between daily recordings, individual animal period was used as the experiential unit for evaluating the animal responses, as in most indoor feeding trail.

However, the levels of PMR intake recorded and estimated grass intake were similar in the two treatments. Studies by Atkins et al. (2020) and Motupalli et al. (2014) have shown that cows prefer to eat PMR when offered it ad libitum, so herbage intake was expected to be a secondary source of feed in the present study. According to Mayne and Wright (1988), silage supplementation can lower herbage intake, which was observed in the present study. Dairy cows typically orientate their intake selection toward the higherenergy components in a mixed ration, due to higher digestibility (Miller-Cushon & DeVries, 2017). Moreover, when PMR is offered in combination with pasture, cows may wait for access to the PMR instead of seeking alternative feed while on pasture (Atkins et al., 2020). A high proportion of concentrate in the diet is also reported to reduce herbage intake (Bargo et al., 2003; Tozer et al., 2004). In the present study, the concentrate DM proportion was 40% of TDMI. Thus, to increase the proportion of fresh herbage ingested in a PTG system, the amount of PMR offered should be restricted, as suggested by Dall-Orsoletta et al. (2016) and Civiero et al. (2021).

In a recent meta-analysis of PTG systems, Molle et al. (2022) examined the effects of access time to pasture in PTG on feeding behaviour and feed intake by different ruminant species and concluded that there is no restriction on herbage DMI when the pasture access time exceeds 9 h d⁻¹. The DAY and NIGHT treatments in the present study had access to pasture for 10 and 12 h, respectively, and thus had scope for high herbage intake. In addition to pasture access time, pre-grazing sward height can influence herbage intake on pasture (Bargo et al., 2003). In the present study, pre-grazing compressed sward height was 20 cm and pre- and post-grazing sward height differed from those for ryegrass swards reported by e.g. Phelan et al. (2013) and Ganche et al. (2014). This is due to lower tiller density (Virkajärvi, 2004) in Scandinavian pastures due to different botanical composition. Higher pre-grazing height allows cows to select the best-quality herbage within a sward, and according to Johansen and Höglind (2007) the post-grazing sward height under Scandinavian conditions should not be below 9 cm to maximise herbage intake and milk yield. The postgrazing sward height in our study was 13 cm, indicating that sward height was not a limiting factor and that the animals had good herbage intake conditions.

A study by Soriano et al. (2001) found higher DMI in cows grazing after the evening milking compared with after the morning milking, while Sairanen et al. (2006) observed a trend for higher herbage intake in cows grazing during night-time at high latitudes. These findings were not confirmed in the present study. The magnitude of the differences in intake variables may in fact show that DAY-grazing cows have a higher (d >0.8) herbage intake potential than NIGHT-grazing cows.

Milk yield of the cows in our study was comparable to that in a study by Eckert et al. (2018), where cows postpeak lactation were fed a PMR in combination with grazing. In the present study, there were no significant differences in milk yield and milk composition between the cows fed ad libitum PMR combined with DAY or NIGHT pasture access.

Due to the beforementioned limitations in the study design, the outcome might have been different under other circumstances. Based on our experiences, we recommend longer adaption and recording periods (several weeks) with larger numbers of animals for future experiments, change-over designs might be appropriate under certain conditions.

Enteric methane emissions

Recording enteric CH₄ emissions from dairy cows in mixed feeding systems is challenging, as emissions are related to feed intake and diet digestibility (Ramin & Huhtanen., 2013), why a sufficient adaptation period is necessary to get reliable results. Even though the registration period in this experiment was only seven days, the adaptation period comprising of 25 days was a relatively long period as recommendations for adaptation periods in digestion trials fall in the range of 10-14 days (Cochran & Galyean., 1994).

The absence of significant differences in TDMI and milk yield observed for the DAY and NIGHT groups was reflected in similar enteric CH₄ emissions, CH₄ intensity (g kg ECM⁻¹) and CH₄ yield (g kg DMI⁻¹). Total CH₄ emissions (from combined GEM) were consistent with other European values (range 251–498 g d⁻¹, mean 376 g d⁻¹) reported in a meta-analysis by Appuhamy et al. (2016). The CH₄ emissions under PTG conditions have previously mostly been recorded using the sulphur hexafluoride tracer technique (SF₆) (e.g. Dall-Orsoletta et al., 2016; Civiero et al., 2021), with only two trials using the GEM system (reports jaarrapport 1: 2020 and jaarrapport 2: 2021 from the Wageningen Livestock Research Institute).

Dairy cows in a previous PTG experiment, with similar TDMI and DMI proportions between indoor and pasture, produced more CH₄ (+109 g d⁻¹; Civiero et al., 2021) than the animals in our study. This difference could be explained by differences in PMR formulation and silage and herbage quality, combined with different CH₄ measuring techniques (SF₆ vs GEM). Koning et al. (2022) investigated CH₄ emissions over two years from cows in a mixed feeding system (indoor silage plus grazing) using two GEM units (indoor and outdoor) and found similar values of 371 and 379 g d⁻¹ cow⁻¹ over the years which are similar to the values obtained in the present study.

The CH₄ emission values for the two groups per GEM unit (Figures 1 and 2(A), Table 4) indicated significantly lower emissions on pasture than indoors. The emissions per GEM unit were not significantly different between the grazing treatments but differed significantly between the GEM unit in both treatments. The CH₄ emissions recorded indoors were consistent with findings by Ramin et al. (2021) in the same research facility (hourly emissions of 16-21 g h^{-1} or 384-504 g d^{-1}), while the outdoor CH₄ emissions were consistent with values found by Waghorn et al. (2016) for cows on pasture $(10-15 \text{ g h}^{-1}, 240-360 \text{ g d}^{-1})$. Our findings indicate potential of fresh grass inclusion in the diet to reduce CH₄ emissions over a short period. We observed a decrease in CH₄ emissions of approximately 28% when the cows were moved from barn to pasture. A similar difference was observed by Koning et al. (2022) in a mixed feeding trial (LONG vs SHORT treatment), while Denninger et al. (2019) reported an increase (+30%) in emissions when cows were moved from summer pasture to winter barn.

The lower CH₄ emissions from cows grazing compared with cows eating conserved forage may be explained by factors related to herbage quality, e.g. higher sugar content and organic matter digestibility, lower NDF and crude fibre content (Koning et al., 2022). Pasture or grazing management can influence herbage quality and many strategies can be used to reduce CH₄ emissions, as shown by Juan Vargas et al. (2022). At high latitudes it may not be feasible to rely solely on grazing during the summer, but our results indicate that even a small inclusion of grass in the diet can reduce daily CH₄ emissions. More research is needed to identify the mechanisms by which fresh grass fermentation reduces CH₄ emissions in mixed feeding systems. The adaptation of the rumen during a repeatedly rapid change of diet (silage and fresh grass) and its impact on CH₄ are also worth exploring. GEM units in a part-time grazing system

Recordings of CH_4 emission when animals are alternating between different environments within a day

are difficult as few experimental techniques can follow the diurnal patterns and measure short term effect in grazing condition. Consequently, experiments on CH₄ emissions from PTG systems are rare, due to this difficulty, and only two sources mention the simultaneous use of indoor and outdoor GEM units (Klootwijk et al., 2021; Koning et al., 2022). To our knowledge, the present study is the first article to use the method in an experiment comparing grazing treatments. Using a combination of two GEM units, one on pasture and one indoors, provided the potential to record CH₄ emissions in the complex experimental feeding system with more accuracy.

The GEM unit is a spot-sampling technique that

requires a minimum of 20-30 voluntary visits per cow and treatment to significantly detect an effect, equating to 7-14 days of recordings (Renand & Maupetit, 2016). Recording using two units increases the time required to obtain a sufficient number of validated visits (similar numbers of indoor and outdoor visits), with the outdoor unit needs a longer recording period to reach the same number of visits as the indoor unit. According to Waghorn et al. (2016) and Hammond et al. (2016), some animals avoid visiting the GEM units without explanation, and this happens more frequently with grazing animals. Despite the relatively long training in our study (more than three weeks) that was needed in order to get enough individual visits to the GEM unit on pasture in order to have reliable CH₄ data on pasture, six cows never learned to visit the GEM unit freguently enough. This caused an imbalance in the total number of observations between the two treatments and reduced the power of the statistical evaluation. In the present study, the distribution of visits during 24 h was more balanced within the NIGHT than the DAY treatment. To compensate for this, Koning et al. (2022) separated measurements by the indoor and outdoor GEM units, which made it possible to lower the interval between visits and increase the feed quantities offered per visit to the outdoor unit to encourage visiting. Thus individual setting of the indoor and outdoor GEM units should be considered, to improve the validity of the recordings. Based on findings in the present study, the recording period should be extended to a minimum of 14 days to ensure sufficient data (20-30 visits per cow to each GEM unit). Overall, we obtained promising results from the two GEM units connected in different environments (indoor and pasture), which could improve estimation of CH₄ emissions from cows in mixed feeding systems. However, longer-term experiments and a more complex experimental design (e.g. change-over design) are needed to confirm the findings of this study.

Animal behaviour and diurnal patterns of grazing

Use of a behaviour recording device (Nedap) in this study allowed us to investigate the possibility that the lower CH₄ emissions recorded by the outdoor GEM unit were caused by lower feed ingestion. The recordings demonstrated that the DAY and NIGHT treatment were actively engaged in grazing outdoors during the pasture access time and visited the GEM unit over the same hours (Figures 1 and 2(B)). Therefore, it is unlikely that the lower CH₄ emissions outdoors were solely due to low herbage intake.

Due to the high latitude at the study site, the cows were not exposed to full darkness, but to four hours of civil twilight per day. The cows in the NIGHT treatment grazed actively until start of the twilight when released to pasture after milking. During the twilight hours, they engaged in other activities such as rumination or resting, which is consistent with findings by Gibb et al. (1998) that cattle avoid grazing at midnight. In agreement with Kismul et al. (2019), we found no circadian eating rhythm related to eating events at dawn and dusk, and instead we observed a grazing peak when cows entered the paddock.

In farmed animals, the natural grazing pattern is artificially modified by farm management routines such as milking, pasture access, indoor feeding etc. According to Molle et al. (2022), when pasture is offered repeatedly at the same time of the day, this meal becomes a time marker. We observed that delivery of fresh PMR and time of pasture access acted as time markers, with a high proportion of each cow's time dedicated to eating indoors and outdoors immediately after each milking event end. This effect was even more pronounced when cows were moved onto pasture (>50% time dedicated to grazing) immediately after milking, and especially with evening pasture access (NIGHT treatment).

The major nutritional needs of the cows in this study were satisfied in indoor feeding so the observed high proportion of grazing activity, but low grass consumption, indicates that pasture acted as a valuable resource for the cows on other aspects apart from nutritional value. This is similar to findings by Charlton et al. (2013) that cows engage in grazing activities even when they have no nutritional need to forage.

Conclusion

This study investigated animal performance, enteric CH₄ emissions and behaviour in dairy cows in two part-time grazing systems (daytime and night-time pasture access) in Northern Sweden. Enteric CH₄ emissions in the mixed indoor-outdoor system studied were measured by connecting two GEM units in different environments.

Day or night-time grazing treatment showed no statistical differences on estimated herbage or PMR intake, milk production or enteric CH₄ emissions. However, there was a rapid shift in recorded CH₄ emissions between the indoor and outdoor settings, with CH₄ emissions on pasture being significantly lower (28%) than those indoors. Under the feeding strategy employed (ad libitum PMR and ad libitum herbage allowance), cows oriented their consumption towards the indoor feed, regardless of time of access to pasture. Cows also showed a high proportion of grazing activity despite a low nutritional requirement remaining after indoor feeding, indicating that cows are willing to graze even when they are predominantly fed indoor. The use of two GEM units allows rapid, short-term variations in CH₄ emissions to be recorded over the 24 h of a day in a multiple feeding system. Several GEM units' method can improve the recording of CH₄ emissions in mixed feeding systems by considering the emissions from each environment. Further studies involving multiple GEM units with dairy cows fed from multiple sources should be carried out to validate the method.

Acknowledgements

The authors wish to thank the farm technicians and students at SLU Umeå. We are grateful for the assistance of Dr. Rebecca Danielsson and Associate Professor Horacio Gonda from SLU Uppsala. We also thank Maria Akerlind for her valuable assistance. The study was financially supported by Trøndelag county authority, Steinkjer municipality, Norwegian Institute of Bioeconomy Research (NIBIO) and the Swedish University of Agricultural Sciences (SLU). This study was an activity at the Grazing Living lab within the project SustAinimal (SLU, Umeå, Sweden).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Trøndelag county authority, Grant/Award Number: RT 21.05; Steinkjer municipality, Grant/Award Number: 2019/198; Norwegian Institute of Bioeconomy Research (NIBIO), Grant/ Award Number: 19/00310-14 project 51516; SustAinimal, funded by the Swedish Research (SLU), Formas Council, Grant: 2020-02977.

Data availability statement

The data that support this paper are available from the corresponding author upon request.



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