

## DOCTORAL THESIS NO. 2025:05 FACULTY OF VETERINARY MEDICINE AND ANIMAL SCIENCE

# Grazing at high latitudes

Impact of grazing on dairy cows' performances and enteric methane emissions in Norway and Sweden

QUENTIN LARDY



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## **Quentin Lardy**

*Faculty of Veterinary Medicine and Animal Science Department of Applied Animal Science and Welfare*  Umeå



**DOCTORAL THESIS**

Umeå 2025

Acta Universitatis Agriculturae Sueciae 2025:5

Cover: The methane archipelago (CH4) Olivier Martin. (2024). Original work. olivmartin.com

ISSN 1652-6880

ISBN (print version) 978-91-8046-440-6

ISBN (electronic version) 978-91-8046-490-1

https://doi.org/10.54612/a.1nrmmqm3vh

© 2025 Quentin Lardy, https://orcid.org/0000-0002-9963-8439

Swedish University of Agricultural Sciences, Department of Applied Animal Science and Welfare, Umeå, Sweden

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Print: SLU Grafisk service, Uppsala 2025

## Grazing at high latitudes. Impact of grazing on dairy cows' performances and enteric methane emissions in Norway and Sweden

### Abstract

The intensification of the dairy industry has led to less reliance on grazing as a primary animal feed source in both Norway and Sweden. The livestock sector is also under considerable societal pressure to reduce its climate footprint. Indeed, enteric methane is an important source of greenhouse gas emissions and there are ongoing investigations to find mitigation strategies.

The aim of this thesis was to assess various grazing management systems with different levels of herbage intake in a cow's diet. Implemented as a collection of four papers, this thesis investigates dairy cows' performances (intake, milk production), behavioural responses, and enteric methane emissions as effects of their consumption of herbage on pasture and grass silage indoors. Two GreenFeed units, both indoors and outdoors, were employed to investigate enteric methane emissions in part-time grazing systems.

This thesis highlights that energy corrected milk remains at the same level with a high proportion of herbage intake as compared to indoor feeding during the summer in Norway and Sweden. The implementation of minor changes in grazing management, such as aligning the provision of fresh pasture with the animals' behavioural preferences, yields promising results if grass intake remains the main source of feed, as the enteric methane emissions recorded from cows fed fresh grass on pasture were significantly lower (20-28%) compared to cows fed silage indoors. This underscores the possibility to use grazing as an enteric methane mitigation strategy.

*Keywords:* behaviour, dairy cow, grazing, pasture, mitigation, herbage intake

*Author's address*: Quentin Lardy, SLU, Department of Applied Animal Science and Welfare, SLU, 901 83, Umeå, Sweden. Email: quentin.lardy@slu.se

## Bete på nordliga breddgrader. Påverkan av bete på mjölkproduktion och enteriska metanutsläpp i Norge och Sverige

## Sammanfattning

Intensifieringen av mjölkproduktionen har lett till att bete används allt mindre som primärt fodermedel i både Norge och Sverige. I tillägg läggs allt större fokus på att husdjursproduktionen, med fokus på idisslarna, ska minska sitt klimatavtryck. Enterisk metan från idisslare är en viktig källa till utsläpp av växthusgaser och flera studier pågår för att hitta strategier för att minska dessa.

Syftet med denna avhandling var att utvärdera betessystem med olika nivåer av betesintag i foderstaten för främst mjölkkor. Avhandlingen är genomförd som en sammanläggning av fyra artiklar, med fokus på foderintag, mjölkproduktion, beteende samt kornas metanutsläpp som respons på konsumtion av bete respektive gräsensilage inomhus. För att mäta metanutsläppen användes två GreenFeedenheter, både inomhus och utomhus, för att undersöka enteriska metanutsläpp i betessystem med korna på bete under delar av dygnet.

Studierna i denna avhandling visar att produktionen av energikorrigerad mjölk kan bibehållas på samma nivå med ett högt gräsintag i mjölkkornas diet under sommaren jämfört med inomhusutfodring av ensilage. Implementering av mindre förändringar i betesskötseln, som att anpassa tiden på dagen då korna erbjuds nytt bete efter djurens beteendepreferenser, gav lovande resultat för att vidmakthålla bete som det huvudsakliga fodermedlet. De metanutsläpp som uppmättes från kor som betade var betydligt lägre (20-28%) jämfört med kor som utfodrades med ensilage inomhus. Detta understryker möjligheten att använda bete som en strategi för att minska enteriska metanutsläpp.

*Nyckelord*: beteende, mjölkko, bete, betesmark, minskning, gräsintag

*Författarens adress:* Quentin Lardy, SLU, Department of Applied Animal Science and Welfare, SLU, 901 83, Umeå, Sweden. Email: quentin.lardy@slu.se

## **Dedication**

To Gary, my unwavering champion and steadfast supporter throughout this PhD journey.

"Remember, no matter how slow you go, just keep yourshell motivated and your pace steady, anything takes time, especially a PhD journey!" **Gary Slitherwell**



The methane archipelago (CH4), original illustration from Olivier Martin

# **Contents**





## List of publications

This thesis is based on the work contained in the following papers, referred to by roman numerals in the text:

- I. Lardy, Q., Ramin, M., Lind, V., Jørgensen, G., Höglind, M., Ternman, E., & Hetta, M. (2023). 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.*
- II. Lardy, Q., Ramin, M., Hetta, M., Jørgensen, G. H. M., & Lind, V. (2024). Predicted methane production from Italian ryegrass pastures with contrasting chemical composition under sheep grazing in Northern Norway. *Journal of Science Food and Agriculture Reports, 4(8), 316-322*.
- III. Ternman, E., Lardy, Q., Danielsson, R., & Gonda, H. (2024). Providing fresh pasture in the afternoon for full-time grazing dairy cows increases energy-corrected milk yield*. Journal of Applied Animal Behaviour Science, 106477.*
- IV. Lardy, Q., Kismul, H., Nyamuryekung'e, S., Sandvik, J., Hetta, M., Ramin, M. & Lind, V. (2025). Comparisons of milk production, methane emissions and time allocation in dairy cows kept part time on exercise or grazing pasture in an automatic milking system*. Journal of Dairy Science, submitted on 30/11/2024.*

All published papers are open access.

The contribution of Quentin Lardy to the papers included in this thesis was as follows:

- I. Involved in planning the practical study. Shared responsibility for conducting the experiment. In charge of the data management and performed the statistical analysis. Wrote the manuscript with regular input from the supervisors. Revised the manuscript under supervision.
- II. Involved in planning the practical study. Re-designed equipment to perform the study. In charge of the *in vitro* experiment under the guidance of supervisors. Shared responsibility for conducting the experiment. In charge of the data management and performed the statistical analysis. Wrote the manuscript with regular input from the supervisors.
- III. Shared responsibility for conducting the experiment and formal analysis. In charge of the behaviours data curation and figures. Participated in writing the manuscript, reviewing, and editing.
- IV. Involved in planning the practical study. Shared responsibility for conducting the experiment. In charge of the data management and performed the statistical analysis. Wrote parts of the manuscript with regular input from the supervisors.

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"For most of history, man has had to fight nature to survive; in this century he is beginning to realize that, in order to survive, he must protect it." **Jacques-Yves Cousteau**

"Supposedly Cousteau and his cronies invented the idea of putting walkietalkies into the helmet. But we made ours with a special rabbit ear on the top so we could pipe in some music."

### **Steve Zissou**

from The Life Aquatic with Steve Zissou by Wes Anderson et al. (2005)

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Photo: Horacio Gonda

# Abbreviations





## 1. Introduction

Grasslands comprise 26% of the world's total land area and 80% of the agricultural land, thereby encompassing a diverse range of ecosystems (Steinfeld, 2006). The world's population is set to continue rising, estimated to approach 10 billion by 2080 (United Nations, 2024). This will require a corresponding rise in food production to meet the rapidly growing population needs and this challenge is even greater given that in 2015, undernourishment had become a widespread issue, affecting around 800 million people globally (FAO, 2015). Animal-sourced food contributes to 18% of global calorie consumption and 25% of global protein intake (FAOSTAT, 2016). The demand for animal products is surging in many regions worldwide, driven by increasing incomes, growing population, and urbanisation. Between 2010 and 2050, the global demand for meat and milk is expected to increase by 73% and 58% (FAO, 2011), respectively. However, the natural resources required to meet these demands are limited (Gerber et al., 2013). In certain regions of the world, arable land is scarce, thus increasing the reliance on a biological process that transforms otherwise inaccessible protein sources into food that is suitable for human consumption.

Humans cannot digest cellulose, which consequently prevents us from accessing most of the energy within grasses. However, this process does occur within ruminants, named after their defining digestive feature, the rumen, wherein a complex microbiota enables the digestion and fermentation of cell walls, such as fibre. This capability allows them to efficiently utilise energy from sources that would be otherwise unusable by monogastric livestock, non-human edible sources of food. Ruminants require 0.4 kg less human-edible feed to produce 1 kg of meat, compared to the required amount in monogastric systems (Mottet et al., 2022). These figures can be significantly reduced in grazing systems, where the need for humanedible feed can be largely decreased.

Ruminants' natural behaviour of grazing, that is, harvesting grass to meet their nutritional needs, has been exploited since the beginning of their domestication. This practice has formed the bedrock of ruminant livestock agriculture for centuries. However, recent intensification within the livestock sector has led to a significant decline in the utilisation of grazing in Europe (Van den Pol-van Dasselaar et al., 2020). With growing consumer concerns regarding animal welfare, local production, and sustainability of food production, the debate around the necessity of grazing livestock is regaining attention. In addition to the pressing issue of securing the production of sufficient food for the growing population, the food industry faces an escalating array of challenges. The most crucial of these is reducing the impact of man-made climate change. A notable by-product of rumen microbial fermentation is the emission of methane  $(CH<sub>4</sub>)$ , a potent greenhouse gas  $(GHG)$ . These emissions constitute a significant portion of the agricultural sector's GHG emissions in most countries and therefore must addressed.

This thesis emerges from a collaborative Swedish and Norwegian research project aimed at addressing pertinent issues within the dairy and grazing research field. It primarily focuses on evaluating the animal performance of various grazing systems for dairy cows and their potential impact on intake, milk performances, animal behaviour, and enteric methane (eCH4) emissions within a Scandinavian context.

## 1.1 Grasslands: the keystone of grazing

### 1.1.1 Grassland origins in Europe

Natural grasslands were rare in post-glacial Europe (22 000 to 14 000 years ago), covering about only 5% of the region until the early Neolithic period (Hejcman et al., 2013). The majority of current grasslands are the result of human activity, classifying them as secondary vegetation that precedes original forests and open woodlands. European grasslands, or pastures, can be categorised (Hejcman et al., 2013) based on the extent of human intervention to maintain their presence in the landscape.

Natural grasslands are predominantly shaped by environmental factors, such as arid conditions in steppe regions or low temperatures and short growing seasons. These grasslands exist with minimal or even a complete absence of human influence (Hejcman et al., 2013). As human activity increased, particularly with the advent of agriculture during the Mesolithic-Neolithic transition (4000 – 3000 BC), seminatural grasslands began to develop. These areas are shaped by human land use, with practices such as grazing and mowing playing a critical role in their maintenance (Hejcman et al., 2010). Additionally, temporary grasslands have emerged through modern agricultural practices, characterised by its high productivity. In this context, human intervention is significant; practices such as fertilisation, irrigation, and selective breeding are employed to enhance both forage quality and quantity (Pavlů et al., 2011).

Grassland management has been a tool for many centuries to enhance biomass and animal production. The first written traces in Europe come from Roman texts, such as Marcus Porcius Cato (244 BC) (Hooper & Ash, 1934), and Lucius Junius Moderatus (4 AD) (Ash, 1941), which provide evidence of early methods used to improve pastures, including practices such as resowing, manure fertilisation, and the introduction of legumes to enhance soil fertility and forage quality. These writings demonstrate the historical significance of grasslands in Europe and the early grassland management practices aimed at optimising fodder production.

### 1.1.2 Overview of European grasslands

Forage remains the primary feed resource for ruminants in Europe, whether provided as pastures, forage crops, or preserved as hay, silage, or haylage. This production of forage occurs on different agricultural lands, however, the distinction between these different grasslands is often unclear. The European Commission defines permanent grasslands (natural and semi-natural) as "land used to grow grasses or other herbaceous forage naturally (self-seeded) or through cultivation (sown), and that has not been included in the crop rotation of the holding for five years or longer" (Commission Regulation, EC, No 796/2004). Permanent grasslands can serve a dual purpose: they provide grazing areas for livestock and produce harvestable forage. Conversely, temporary grassland refers to arable land used for forage production over a short-term period (less than 5 years), as part of a crop rotation system. These grasslands, also called leys in Sweden or production pasture in Norway, are primarily harvested (they can also be grazed), and often involving a greater degree of mechanisation and inputs.

A large part of European area is devoted to forage production. According to Eurostat (2020), approximately half of the European Union (EU)'s land is farmed and the main type of farming land cover is arable land with 62 % of utilised agricultural area (UAA). Permanent grasslands and meadow cover a further 30 % and the rest is permanent crops and kitchen gardens. One fifth of the arable land is dedicated to the production of forages. In total, the proportion of fodder area (area dedicated to the production of forages) in the UAA accounted for 42.2% in 2020. However, the type of fodder production, the portion of UAA allocated, and its significance exhibits considerable variation among countries and even within countries. These numbers highlight the importance of the grasslands, and therefore ruminant production, across European countries.

The proportion of agricultural land used as grassland differs between European regions (Lee, 1988) (Figure 1, Panel A). On average, temporary grassland constitutes about 10% of the total European grassland area. Permanent grasslands are particularly prevalent in mountainous regions in Central Europe (Alpes, Massif Central, and Pyrenees) and in Western Europe (Ireland, England, certain regions of France, and Portugal) where they cover more than 50% of UAA. In the Mediterranean regions, grasslands account for a significant portion of land use (30 to 40% of the UAA) and are primarily grazed by sheep and goats. In the Nordic countries, permanent grasslands make up only a small fraction of the UAA (less than 3%), but the percentage of the temporary grasslands is rather impressive with some regions of Scandinavia having more than 80% of UAA dedicated to fodder production (Figure 1, Panel B). In the lowlands, grasslands comprise a small amount with around 20-25% of the UAA with some exceptions such as Utrecht in the Netherlands ( $> 80\%$ ) and the Puszta in Hungary ( $> 60\%$ ).



Figure 1. Type of agricultural land use (Panel A) and fodder production area (Panel B) in percentage of share of UAA (%) in Europe in 2020 (Source: Eurostat, 2020).

## 1.2 Let's graze!

A key element in the maintenance of grasslands is regular harvesting. Without multiple defoliations per year, these areas (except natural grassland) would quickly revert to their climax vegetation state. This defoliation can be achieved through grazing by wild or domesticated ruminants or via human activities such as mechanical harvesting. For the purposes of this thesis, the focus will be on the dairy sector and grazing, thereby encompassing both the natural feeding behaviour of herbivores consuming grasses and forbs, and as a management strategy in animal husbandry.

#### 1.2.1 The grazing systems

A grazing system is defined as "an integrated combination of animal, plant, soil, and other environmental components, and the grazing methods and management objectives designed to achieve specific results or goals" (Allen et al., 2011). This involves monitoring and controlling the productivity and quality of pastures while also managing the various factors related to animal access and their natural harvesting capacity.

Defining the different feeding systems is important as it will influence the grazing system and both management choice and importance. One of the most widely recognised classification methods is that of Seré et al. (1996), which categorises livestock production into three broad feeding systems: mixed, indoor, and grazing. The first category is the mixed crop-livestock system, which produces most of the world's ruminant meat and milk. In this category, until 90% of the feed dry matter (DM) intake comes from forage (conserved or fresh), with the remaining coming from crop residues, by-products, or feed grains. Integration with crop production can vary across scales, and external feed inputs are common. The second category, landless systems, often referred to as grain-fed or intensive systems, involves highly industrialised operations with less than 10% of the feed produced on the farm, and high stocking densities. Even within landless systems, ruminants may spend part of their early life grazing or be fed grass in the form of silage. The third category, grazing systems, exclusively applies to herbivores and is characterised by more than 90% of the feed coming from temporary or permanent grasslands (mostly grazed), forage crops, with some supplementation. In the European dairy sector, all three livestock production categories are used, though only mixed crop-livestock systems and grazing systems incorporate grazing.

These systems employ a wide range of grazing management strategies, each with different names and approaches. However, most can be analysed through two core factors: defoliation intensity and defoliation frequency (Carvalho, 2024; personal communication). The first factor determines the amount of biomass removed and the second the frequency of grazing session on a given area. In intensive grazing dairy barns, the most common system is characterised by high defoliation intensity and low defoliation frequency. This system is known as the rotational grazing system, with many iterations such as high-intensity low-frequency grazing, strip grazing, short duration grazing, time-controlled grazing, and cell grazing among others. The key characteristic of this system is that the herd is rotated between different fields or paddocks according to a set schedule. This enables the efficient utilisation of available biomass with minimal selection by the animal. The low frequency is essential to allow pastures to recover before the next grazing session. Across various ruminant production systems, the most widely used grazing approach is the continuous grazing system, characterised by both high intensity and high frequency (Teague et al., 2011).Within this grazing strategy the animals have continuous, unrestricted access to the grassland throughout the year, or for a set time-period. Thus, how frequently and intensely a particular plant or area of the grassland is grazed is entirely dependent on the livestock. Another form of continuous grazing combines low intensity with low frequency and is often linked to extensive practices, such as pastoralism, or reserved for non-productive animals. Pastures in these systems are typically unmanaged and located in low productivity but ecologically significant areas. Many rewilding projects use this grazing strategy through ruminant reintroduction. The last combination is low defoliation intensity with high defoliation frequency, focusing on animal preferences as the management target. This aim of this approach is to harvest only the upper layer of the pasture (low defoliation) but frequently expose the pasture to animal harvesting. This strategy encourages animals to selectively graze high-quality forage and promotes rapid pasture regrowth, making it ready for subsequent grazing sessions. Some of the recent versions of this grazing system are also known as Rotatinuo (Marín et al., 2017) and Nieuw Nederlands Weiden (Philipsen & Van den Pol-van Dasselaar, 2018). The grazing management strategy in mixed crop-livestock farming is largely shaped by the proportion of grass intake that the farmer aims to achieve. Each system is tailored to suit specific environmental conditions, available resources, and the individual knowledge and philosophy of the farmer.

### 1.2.2 Grazing in Europe

The importance of grazing as a source of nutrient intake can vary greatly within the dairy sector. One of the limiting factors is the capacity to provide both good quality and sufficient quantity throughout the year. Certain regions in Europe with an all year-round or extended vegetative season, can utilise grazing during most seasons of the year. In these regions, grazing is generally the preferred method of animal feeding. In Northwestern Europe, such as Ireland, grazed pasture is the largest component of a cow's diet, representing 82% of the total dry matter intake (DMI) (O'Brien et al., 2018). In most other regions, grazing is seasonal and indoor winter feeding is mandatory. During the summer months, grazing is the main feeding strategy and the production of conserved forage for winter feeding is crucial. This system can be found throughout Europe. In regions with short summers, farming often follows a part-time grazing strategy, providing livestock with both indoor silage and outdoor grass. This approach is common in most Scandinavian countries, where relying exclusively on grazing during the summer is rare.

Data on grazing in Europe is relatively scarce, and reported data is often not standardised, making it difficult to compare or combine data sources (Van den Polvan Dasselaar et al., 2020). The survey by Van den Pol-van Dasselaar et al. (2020) among members of the Working Group "Grazing" of the European Grassland Federation provided valuable insights into dairy cow grazing across Europe. Figure 2 shows that Scandinavia, Western Europe, and Switzerland are, to a large extent, utilising grazing practises in their dairy systems, with more than 50% of dairy cows allowed to graze for at least part of the year. In Central Europe (Denmark, Germany, and Austria), less than 50% of the dairy cows are allowed to graze. Both Eastern and Southern Europe display a very low number of dairy cows grazing, apart from Lithuania. The definitions of grazing can differ across countries and the perception of what grazing mean also fluctuate within society: outdoor access, feeding strategy, time on pasture.



Figure 2. Visualisation of grazing dairy cows (% dairy cows) in Europe from the European Grassland Federation data (Van den Pol-van Dasselaar et al., 2018) and complementary information on Iceland (same welfare legislation as Norway), and Slovakia (Pastierik et al., 2023).

### 1.2.3 Understanding the decrease in dairy cow grazing

Globally, dairy production predominantly relies on non-grazing systems, with grazing-based milk production accounting for only 10 to 15% of the total output (Shalloo et al., 2018). Although grazing systems are more prevalent in Europe compared to the other continents, their use is steadily declining (Hennessy et al., 2020; Van den Pol-van Dasselaar et al., 2020). In regions where grazing remains prevalent, production systems typically focus on maximising milk yield (MY) per unit of pasture, contrasting with high-input/high-output systems. The latter are often implemented in areas with limited land availability and/or climate conditions that elevate winter housing costs. Resultingly, there is a shift toward maximising annual MY per cow, which reduces the inclination to maintain grazing. The European Dairy Farmers (EDF) investigated the percentage of cows grazing in six countries in Northwestern Europe (Reijs et al., 2013). Among the countries, the percentage of EDF that applied grazing dropped from 52% in 2008 to 35% in 2012. In the same report, experts predicted that these percentages would continue to decrease until 2025 in North-West Germany, Northern Ireland, the Netherlands, and Denmark. The survey by Van den Pol-van Dasselaar et al. (2020), shows that the Scandinavian region maintains a high level of grazing dairy cows. This could be explained by the animal welfare acts in both Sweden and Norway, stipulating outdoor access for dairy cows during the summer season. However, an important factor is that dairy cows, particularly those in automatic milking systems (AMS), only have access to an outdoor paddock (also called an exercise paddock) and their intake of fresh grass is relatively low (Bergsten et al., 2015).

Despite the benefits of grazing the proportion of grazed grass in European dairy cows' diets is declining as production systems intensify (Hennessy et al., 2020). In much of Central, Eastern, and Southern Europe, as well as in countries such as Denmark, the tradition of grazing dairy cows is either disappearing or has already been lost in non-organic farming systems (Isselstein & Kayser, 2014). Large, modern farms with high-yielding dairy cows may reduce grazing to better control their diet and optimise grassland use. The gradual shift towards greater reliance on conserved forage and concentrate to better control intake and increase milk production has also led to a desynchronisation between calving and the grazing season. Improvements in silage quality and conservation further contributed to this shift. In scenarios where feed supplementation is provided, the grazing time often decreases. Additionally, the increase in herd sizes can further complicate grazing management; as farm sizes increase, the grazing area surrounding the farm remains constant, leading to an increased grazing pressure through higher stocking rates. When additional land is integrated into the grazing area, the distance to the milking parlour can become a limiting factor. Another contributing factor to reduced grazing is the growing adoption of AMS. Whilst it is possible to combine grazing with robotic milking systems (Wiktorsson & Spörndly, 2002), challenges may arise in practice (Parsons & Mottram, 2000).

To address the decline in grazing, several countries, including the Netherlands, Germany, Portugal, France, and Switzerland, have introduced economic incentives from both their private and government sectors. These initiatives aim to promote grazing practices or compensate for ecosystem services that are provided by grazing livestock. In certain European regions, stringent technical standards require a significant portion of DMI to originate from grazing to qualify for certification (e.g., Saint-Nectaire AOP, France). In the case of the AOP Saint-Nectaire, these standards exceed those of France or the broader EU regarding grazing management regulation. To strengthen the link to the terroir and conserve local natural resources, grazing is mandatory for a minimum of 160 days, with tightly controlled complementary feeds. Product quality is prioritised over production volume, which enhances the value of the raw product (milk for cheese) and elevates its market price. This approach remains prevalent in certain European regions where ruminant production is one of the few economically viable agricultural activities. Indeed, it is crucial as these exploitations would struggle to compete against other producers with more favourable environments.

The reduction of grazing can be explained by the decline in grassland areas, the reduction of farms, and the diminishing use of grazing as a feeding strategy. The area of European grasslands has significantly decreased over the past 30 years (Huyghe et al., 2014). According to the third report of the EU MAES initiative (Maes et al., 2015), between 2006 and 2012, the primary drivers of this decline included the conversion of grasslands into arable crops (notably maize, including for biogas production), which accounted for 32% of the lost area; the expansion of urban areas, economic sites, and infrastructure (30%); and the withdrawal from farming (17%). Benoit and Mottet (2023) have noted the increased competition between grasslands and arable crops for energy production, with the former often losing out. At the EU level, natural grasslands are among the most rapidly deteriorating habitats, with 75% of the 126 recognised grassland habitats assessed as being in poor or bad condition. The main threat to these habitats is the abandonment of agricultural management, leading to overgrowth and habitat degradation (European Environment Agency, 2020). The withdrawal from farming is currently one of the greatest challenges of European agriculture and it directly affects the maintenance of grassland. Between 2005 and 2020, the number of farms in the EU decreased by almost 40%, forcing approximately 5.3 million farmers out of business (Eurostat, 2020).

In Scandinavia, it is common for farmers to adopt year-round calving practices. Many even align the peak of lactation of a significant number of cows with the availability of the first cut silage, typically in late autumn or early winter, rather than with herbage growth. This shift reflects the growing adoption of AMS and the reliance on stored feed rather than fresh feed. Animal selection programmes have primarily focused on improving productivity, often neglecting traits that enhance grazing suitability. High genetic merit breeds, optimised for indoor, highconcentrate feeding systems, are generally less adapted to outdoor pasture-based environments (Delaby et al., 2018). Consequently, there is a growing need for more robust animals that can thrive in grazing systems. Selection programmes that prioritise high-concentrate diets often produce animals that are unable to fully express their genetic potential for milk production when placed in grazing systems (Buckley et al., 2005; McCarthy et al., 2007).

### 1.2.4 The reasons to graze

The promotion of grazing as a feeding management practice in dairy systems is not merely a nostalgic nod to traditional farming methods, but rather a valuable management system with significant social, economic, and environmental benefits. Grazing systems, while beneficial in many contexts, can also be challenging to implement and come with notable drawbacks. As production becomes more intensive, many of the ecological, social, and animal welfare benefits associated with grazing tend to decrease. In general, the main drawback from grazing is the higher level of uncertainty and lower level of perceived precision of the system. Table 1 provides an overview of the advantages and disadvantages of different themes highlighted by Van den Pol-van Dasselaar et al. (2020) with the literature associated with each points in the original document. These different themes are presented as a base for discussion rather than an exhaustive list of the services and drawbacks of grazing. They were gathered into *socio-economic* (Labour, Economy and Image), *animal welfare, and productivity* (Animal health and welfare, Grass production and quality, and Quality of the milk), and *environmental* (Biodiversity and Environment) subsections.

#### *Socio-economic*

From a socio-economic perspective, grazing plays a critical role in maintaining rural populations and contributing to the vitality of these areas. Aside from the cultural and traditional bond, it encourages a system based on local resources, increasing local to regional food resilience. As discussed by Benoit and Mottet (2023), the livestock sector is facing increasing competition for arable land, which may lead to a spatial redistribution of livestock production, focusing more on non-arable land and altering ruminants feeding regimes. This could signal a future trend of promoting more extensive dairy production systems that make greater use of roughage and local resources. The utilisation of non-arable land for human edible protein production is important.

Pol	Theme	Advantages	Disadvantages
asselaar et	Animal health & welfare	More possibilities for natural behaviour (e.g., • Less risk of claw problems Lower level of mastitis grazing)	Relatively large fluctuations in the composition of the ration Risk of disease introduction due to infection with specific pathogens (e.g., worms, liver fluke)
Table 1. Advantages and disadvantages of grazing on different themes (source: Van den 2020).	Grass production & quality	mechanically harvested swards thereby reducing • Denser and more persistent swards compared to the requirement for sward renewal	the grassland than mowing and to fluctuating grass supply • Grazing leads to a lower use of the production capacity of • Uncertainty about the availability and quality of grass is a bottleneck for livestock farmers and grass quality
	Quality of milk	• Higher content of unsaturated fatty acids in milk Superior processibility qualities	grass supply and quality. This can impact the production of • Milk quality can be more variable due to the variability of milk products, e.g., cheese yield
	Labour	• Fewer hours of work, because the cow does the work itself	• Management of grazing is more difficult than management of zero-grazing
	Biodiversity	• Grazing leads to more biodiversity	
	Environment greenhouse (minerals, gases)	Less energy consumption · Less ammonia emission $\bullet$ Less CH <sub>4</sub> emissions $\bullet$ Less CO <sub>2</sub> emissions	More nitrous oxide emissions More nitrate leaching More denitrification More N losses
	Economy	cheap means of production; a key factor affecting the economic sustainability of grass based dairy • Grazing involves lower costs; pasture grass is a production is the proportion of grass in the diet	for example reduce the yield potential of grassland resulting Some regions of Europe do not have satisfactory conditions to focus almost exclusively on grass; climatic limitations in an increase in the production costs of forage
	Image	• Cows in the landscape contribute to a positive image of livestock farming	

Table 1. Advantages and disadvantages of grazing on different themes (source: Van den

For example, both Norway and Sweden have vast outfields (utmark), which are comparable to alpine permanent grasslands, and were historically used in dairy production before the Green Revolution. In Norway, for instance, over 45% of the land area consists of outfields which are suitable for grazing. Currently, 40% of these resources are utilised, primarily by sheep, suckler cows, and reindeer (Strand et al., 2019). However, recent analyses indicate that almost half of Norway's outfield grazing potential remains untapped and could be utilised with a higher intensification. According to Rekdal and Angeloff (2021) 11% of the outfield produces feed of very high quality and 42% of good quality. Although transhumance (utilising of outfield) has seen a steep decline, the region remains a core area for this traditional form of farming (in Swedish "fäboddrift" and in Norwegian "setring"). As grass is among the cheapest high‐quality feed sources for efficient ruminant meat and dairy production (Van den Pol-van Dasselaar et al., 2018), it is essential to use these areas to maintain sustainable animal production. Another advantage of a pasture-based dairy system is the reduction of production costs, which usually fall as the proportion of grass fed increases (Ramsbottom et al., 2015). As the focus has been on intensification in the last decades, many farmers and advisors have lost knowledge about grazing, making the reintroduction of such systems challenging. Grazing management is often viewed as more stressful option due to uncertainties about key factors such as intake, biomass growth, and weather conditions. Additionally, it can be viewed as labour-intensive compared to other systems.

#### *Animal welfare and animal productivity*

From a societal point of view, there is a consumer concern about dairy cows' access to the outdoors (Ellis et al., 2009) and pasture access is often mentioned as being an important aspect in cow welfare (Schuppli et al., 2014). This concern from consumers and various stakeholders (Van den Pol-van Dasselaar et al., 2020) has been strengthened by scientific research, which has confirmed the importance and relevance of pasture access on cow health and welfare (Table 1). However, whilst many studies have confirmed cows' preference for pasture access (Arnott et al., 2017; Von Keyserlingk et al., 2017), in certain systems, animals may be more exposed to climatic extremes whilst on pasture (Moons et al., 2014). Cows are particularly sensitive to heat, therefore during hot weather conditions, they often prefer to stay indoors, seek out shade, (Spörndly et al., 2015), or visit the pasture during cooler night-time hours (Charlton et al., 2013; Smid et al., 2018). Along with climatic conditions, infrastructure also plays a critical role, as walking distance is increased on pasture compared to indoors, and poorly maintained walking tracks can lead to locomotor and claw disorders (Burow et al., 2014). Poor body condition and

metabolic disorders (Crossley et al., 2021) can also arise due to a less accurate control of feed quality and intake quantity. It is quite challenging to monitor pasture quality, quantity, and animal intake compared to indoor feeding, making it difficult for farmers to provide an adequate energy and protein supply. For high-yielding cows in seasonal grazing systems, diet formulation is particularly challenging. As noted by Van Vuuren and Van den Pol-van Dasselaar (2006), cows that produce more than 28 kg of milk per day require supplemental feeding to meet energy and protein needs. However, this supplementation is not challenging in a sector where one to three occasions (milking) can be utilised for this purpose. Relying solely on grazing can compromise the energy balance of cows (Chilibroste et al., 1997; Melin et al., 2005). This raises the question of whether the animal material used in breeding for modern dairy production is suitable for a sustainable dairy production which also meets consumer demands of high welfare standards. An important aspect to consider is that the improvement of animal welfare can boost productivity, as healthier cows can reach their genetic potential more efficiently, using fewer resources and expending less energy on recovery.

#### *Environmental*

From an environmental standpoint, natural grasslands are often rich in species and provide important habitats for numerous endangered plants and animals (Henriksen & Hilmo, 2015). Grasslands harbour between 2 and 7 times more biodiversity than field crops (Alkemade et al., 2009). One could argue that human harvesting alone could suffice, making grazing less vital. However, mechanisation is not feasible in all grazing areas due to unfavourable terrains or access limitations. Mechanisation also carries environmental costs, including the energy used in manufacturing equipment and the emissions produced by operating machinery. Further, the defoliation from grazing has a more positive effect on conservation values in most grasslands compared to mowing (Tälle et al., 2016). Extensive pasture-based animal husbandry is one of the few agricultural systems that can actively enhance biodiversity within a landscape (Eriksson, 2022). As highlighted in Eriksson's paper, the temperate and boreal grasslands (semi-natural grasslands) possess the greatest small-scale plant species richness on Earth, as observed at the plot-scale level (one or a few square metres) (Wilson et al., 2012). These landscapes also play a crucial role in water retention, erosion control, and run-off prevention (Bengtsson et al., 2019). Permanent grasslands can also have a greater soil carbon content than croplands, aiding in carbon sequestration and thus mitigating GHG emissions (Soussana et al., 2010). Unlike forests, grasslands contribute to carbon capture and storage primarily through carbon sequestration in the soil rather than aboveground

biomass. This occurs through the fixation of atmospheric carbon into soil organic carbon and the carbon stored in plant roots and belowground biomass (Liu et al., 2023). In fact, it has been found that agricultural soils used for cultivation represent a stock of around 50 tons of carbon per hectare (t C/ha) on average, whilst a permanent grassland represents a stock of around 80 t C/ha, equivalent to that of a forest plot (Demarcq et al., 2022). Furthermore, high quality grasses which are highly digestible and contain a high content of water soluble carbohydrate (WSC) can promote lower eCH<sup>4</sup> when ingested compared to grass silage (Koning et al., 2022). Sound grazing practices could lower  $CH<sub>4</sub>$  intensity (CH<sub>4</sub>I) of ruminant production by 55% according to Zubieta et al. (2021). An interesting analysis by Steinshamn et al. (2021) in Central Norway challenges the general assumption that higher concentrate feeding and increased milk production reduce the global warming potential and energy required per kilogram of milk produced, compared with more extensive systems relying on greater herbage intake from pasture. Several studies suggest that grazed grasslands may balance out or even outweigh the negative effects of GHG emissions caused by livestock production (Batalla et al., 2015; Bellarby et al., 2013). It is important to note that the positive effects reported for permanent grasslands can be greatly reduced by intensive pasture maintenance practices (Röös et al., 2017; Smith, 2014). Overgrazing, especially in confined areas, can result in excessive manure build-up, leading to nitrate leaching, increased denitrification, and elevated nitrous oxide emissions. When large numbers of animals are confined to small areas, the nitrogen load from urine and faeces exceeds the soil's capacity to absorb it, resulting in more nitrogen losses through leaching and volatilisation. This is particularly problematic in areas such as Normandy (France) and the Netherlands, where high stocking densities are common. Intensive pasture use and frequent ploughing further exacerbate these impacts, reducing the overall environmental benefits of grazing systems.

## 1.3 Grazing dairy cows in Norway and Sweden

### 1.3.1 Unique conditions

The agricultural landscape of Norway and Sweden is shaped by unique environmental factors that significantly influence farming practices, including dairy production and grazing. The countries' combined latitudes range from 55 to 71 degrees north, resulting in a climate characterised by long winters with either low or no sunlight, followed by a short but intense growing season. These conditions vary significantly according to the latitude. In the far northern regions, winters are extremely long and dark, with minimal daylight for several months. In contrast, southern areas experience shorter winters with more sunlight, even during the cold months. This variation leads to considerable differences in biomass growth conditions across Norway and Sweden. Due to the Atlantic Gulf Stream, the temperatures are relatively high, making them warmer than other areas at similar latitudes, such as Alaska and Siberia. Along with good precipitation all year-round, this promotes an "intense biomass growth" during the vegetative season that lasts between 3 to 8 months, depending on the latitude and altitude. Summers are followed by 4 to 8 months of minimal or no vegetation growth which presents significant challenges for agricultural and livestock production, forcing these countries to adapt their systems.

The topography itself adds to the complexity. In Norway, agriculture is constrained by a fragmented landscape and rugged topography. The country is dominated by mountains (30%) and is dotted with lakes (covering 6% of the land area) and over 50,000 islands along a chaotic coastline. Sweden, although less mountainous, has a significant part of its territory covered with productive boreal forest (70%) and the greatest number of islands on Earth (267,570 islands). The two countries have a small surface of arable land: 3% for Norway and 6.5% for Sweden. Of this arable land, both use a significant portion to produce ruminant feed. Sweden has 45% of its UAA covered with forage crops (Spörndly & Nilsdotter-Linde, 2011) whereas in Norway 45% of the UAA is only suitable for fodder production (Blandford et al., 2015).

#### 1.3.2 Grazing access and grazing intake

Livestock, especially ruminant production, is the bedrock of Sweden and Norway's agriculture. The combined cattle milk and beef production is the most economically important sector in Norwegian agriculture (Knutsen, 2020) and represents one-third of the productive value of the agricultural sector in Sweden (European Commission, 2024). Both countries livestock production has undergone large structural changes during the last decades. Both the total number of dairy cows and the number of farms keeping livestock has declined, resulting in increased herd sizes. The average dairy herd size in Norway is relatively small, with 32 cows per farm in 2023 (Rustad, 2024) and much higher in Sweden with 113 cows per farm in 2024 (Swedish Board of Agriculture, 2024b). The average MY per cow has increased in all the Scandinavian countries during recent decades, underlying a steady intensification of the dairy sector. The fact that silage serves as the primary feed for dairy cows for a

large part of the year has driven the sector to invest heavily in the development and improvement of handling of conserved feeds. This focus on silage has led to a decline in overall grazing knowledge and practices. Norway hasseen a steep increase in the use of AMS and is one of the countries with the most extensive implementation of AMS worldwide (Vik et al., 2019). The latest report from the largest Norwegian dairy cooperative (TINE, 2023) revealed that 63% of the cows were milked in an AMS equipped barn, representing 64% of the milk delivered in 2023. Moreover, the latest report from Växa (Växa, 2024), where 68% of the national herd is registered in Sweden, showed that the adoption of AMS is also high with 40% of herds representing 51% of the dairy cows.

In Sweden and Norway, welfare legislation has substantially influenced dairy farming. In Sweden, all dairy holdings must provide outdoor access, whilst Norwegian laws apply only to tie-stall and new free-stall farms since 2013. These regulations aim to ensure that cattle can express their natural behaviours, but they do not mandate specific pasture contributions to energy supply (at least in conventional production). The development of the "exercise pasture" and "production pasture" is a direct effect of this welfare legislation. These two terms have emerged in Sweden and can also be found in Norway. An exercise pasture is defined as "access to a grass-covered outdoor area of size and quality so that DMI of grazed grass is negligible" and a production pasture is defined as "access to a pasture of sufficient size and quality to allow DMI of grazed grass contributing considerably to daily roughage DMI" (Kismul et al., 2020). The primary goal of an exercise pasture is to allow cows to roam freely and engage in natural behaviours such as walking, resting, or socialising, whilst also maintaining access to their main diet which is provided indoors. Exercise paddocks have predominantly emerged in combination with AMS and are seen by many as grazing management. It is actually closer to an indoor feeding system with access to an outdoor area.

Mandatory outdoor access and grazing face criticism in both countries. Producers argue that they compete in the same market as those from regions where grazing is optional. A report from the Swedish Board of Agriculture (2014) indicated that farmers incur losses by keeping lactating cows on pasture, attributing this to a decrease in MY. Conversely, a Norwegian study (Overrein et al., 2018) found that whilst grazing impacts farm economics, production pasture is often more profitable than exercise paddocks. A Swedish study from Kismul et al. (2018) has also shown that MY does not differ between AMS with an exercise paddock or grazed grass intake yet the authors show in another similar study (Kismul et al., 2019) a reduction in MY but not in energy corrected milk (ECM).

There is little information on dairy cows' herbage intake from pasture in Norway and Sweden. The first Norwegian documentation (unpublished) since the introduction of the welfare legislation in 2013 by Kismul et al. (2020) found that 81% of farmers offered lactating cows' outdoor access in summer, with temporary pastures being more common than exercise pastures. Grazing opportunities varied by milking system, with 86% of manually milked cows provided access compared to 56% in AMS. Recent research by Steinshamn et al. (2021) in Central Norway revealed that grazed grass contributed 5-10% to the total dietary energy intake of the animal. This is influenced by the shorter grazing seasons, but it highlights the low utilisation of grazing as a feeding strategy. Two studies modelling dairy barns in various Norwegian locations (from South to central Norway) had similar estimates with the yearly pasture contribution to the total energy intake of the dairy cows representing 10-17% for Roer et al. (2013) and 3-10% for Bakken et al. (2017). In a survey to grazing dairy farmers in Northern Sweden (Karlsson et al., 2024), pasture intake was not addressed, with the authors noting that most farmers in the study allowed their dairy cows to graze outdoors primarily to comply with legal requirements, and their nutritional needs were being met through other methods. A report using the EDF database from Reijs et al. (2013), which defined grazing as herbage intake on pasture rather than outdoor access or time spent outdoors, shows that the percentage of dairy farmers in Sweden that apply grazing was low (22%) in 2012 and expected to be even lower in 2025 (13%), indicating that fresh grass intake is limited (Figure 3). This figure must be interpreted with caution; however, it shows the low prevalence of grazing and its ongoing diminution in Sweden.



With (partial) grazing Without grazing

Figure 3. Development of the percentage of farms with and without grazing in six countries from the European Dairy Farmers sample (source: Reijs et al., 2013).
In summary, whilst welfare legislation in Norway and Sweden mandates outdoor access for dairy cows, this does not equate to a significant herbage intake. Despite the legal requirements for outdoor access, grazing in these countries often remains a supplementary feeding strategy that is predominantly applied as part-time grazing (PTG). The observation by Van den Pol-van Dasselaar et al. (2020) regarding the scarcity of data on grazing in Europe is also applicable to Norway and Sweden. However, there are still "irreducible Gauls" committed to grazing during the summer months in these countries.

## 1.4 Tackling enteric methane in ruminant production

#### 1.4.1 Methane impacts on climate and sources

Methane is a potent greenhouse gas that plays a significant role in climate change. It is ranked as the second most important GHG after carbon dioxide  $(CO<sub>2</sub>)$ , contributing to approximately 30% of global warming and to 16% of global anthropogenic GHG emissions (Myhre et al., 2013). Whilst  $CO<sub>2</sub>$  can persist in the atmosphere for up to 1000 years, CH<sup>4</sup> has a much shorter atmospheric half-life of 8.6 years (Muller & Muller, 2017). However, CH<sup>4</sup> has a higher capacity to trap heat, making its global warming potential (GWP<sub>100</sub>) 28 times greater than  $CO<sub>2</sub>$  over a period of 100 years (Myhre et al., 2013). The scientific literature proposed a new metric (GWP\*) that improved the method of assessing the actual impact of shortlived climate pollutants such as  $CH_4$  on temperature change. The GWP\* metric not only accounts for the short lifespan of CH<sup>4</sup> but also its atmospheric removal, making it particularly useful for effective climate policy (Lynch et al., 2020). Nonetheless, both approaches agree that CH<sub>4</sub>, with its much shorter atmospheric lifetime, presents a promising target for near-term global warming mitigation.

Methane emissions originate from both natural and human-induced sources. Natural sources (Figure 4) include wetlands, oceans, termites, and geological seepages, whilst anthropogenic sources encompass agriculture, fossil fuel extraction and transport, and waste management (Saunois et al., 2019). Methane from ruminants contributes to approximately 40% of the GHG emissions from beef and dairy production, making it the largest agricultural source of GHG. It also represents about 5% of total GHG worldwide (Herrero et al., 2016).



Figure 4. Global methane budget for the 2008–2017 decade. Both bottom-up (left) and top-down (right) estimates (Tg $CH_4$ /year) are provided for each emission and sink category, as well as for total emissions and total sinks. (source: Saunois et al., 2020).

The average surface CH<sub>4</sub> concentrations are estimated to have increased by 249% from 1750 to 2011, largely driven by human activities (Myhre et al., 2013). This rise is closely linked to the significant growth in global livestock numbers. Over the past decade,  $CH_4$  concentrations have continued to rise (Dlugokencky & Tans, 2024), with more than 80% of the increase between 2010 and 2019 attributed to changes in terrestrial CH<sup>4</sup> emissions in tropical regions (Feng et al., 2022). Currently, CH<sup>4</sup> emissions are unevenly distributed, with tropical regions contributing 64%, middle latitudes 32%, and high northern latitudes (above 60° N) just 4%. Conversely, food and feed imports to Europe have risen significantly since 1990, and the GHG emissions from these imported products are not always counted in national inventory emissions, as is the case in Sweden (Sandström et al., 2018). Thus, a portion of tropical emissions can be indirectly attributed to the Nordic region's food import and emphasises the lack of self-sufficiency in certain food/feed production sectors.

Sweden and Norway exhibit similar trends in their CH<sub>4</sub> emissions associated with agriculture, its importance, and the ongoing reduction trends. In 2018, 8.6% of Norway's national GHG emissions, or 4.5 Mt  $CO<sub>2</sub>$ -eq., came from agriculture (Norwegian Environment Agency, 2020). Since 1990, emissions have decreased by 6.0%, including a 1.3% drop since 2017. In Sweden, agricultural emissions are about 6.4 Mt  $CO_2$ -eq., down 13% since 1990, which is mainly due to efficiency improvements and reduced animal husbandry (Swedish Environmental Agency, 2024). Annual eCH<sub>4</sub> emissions from ruminants are currently around 3.3 Mt CO<sub>2</sub>-eq in Sweden (Swedish Environmental Agency, 2024) and 2.3 Mt  $CO<sub>2</sub>$ -eq in Norway in 2018 (Norwegian Environment Agency, 2020). It represents 51% and 46% of total CH<sup>4</sup> emissions, which account for 6.4% and 4.4% of all Swedish and Norwegian anthropogenic GHG emissions, respectively. In Norway, eCH<sub>4</sub> emissions have fallen by 5% since 1990, whereas in Sweden over the same period, they declined by 10% due to reduced cattle numbers as an effect of higher production per cow (Swedish Environmental Agency,  $2024$ ). It is important to note that CH<sub>4</sub> emissions from manure storage and field spreading represent about 15% of total emissions, however, this aspect is beyond the scope of this work.

### 1.4.2 A quick insight into enteric methane production

Ruminants are key for food security as they convert forages, which are not directly usable by monogastric animals and humans, into animal products for human food. This is possible because of an anaerobic microbial population that is hosted in their digestive tract, but this fermentation process also generates  $eCH_4$  as a microbial byproduct. Most of the eCH<sub>4</sub> has its origin in the rumen  $(\sim 90\%)$ , with the remaining produced in the large intestine (Murray et al., 1976).

Rumen digestion of feed components by the microbiota (bacteria, protozoa, fungi) results in the production of volatile fatty acids (VFA), mainly acetate, propionate, and butyrate used by the animal as a source of energy, and the production of gases  $(CO<sub>2</sub>$  and  $CH<sub>4</sub>)$  which are eliminated through eructation into the atmosphere. Fermentation is an oxidative process, during which reduced cofactors (NADH, NADPH, FADH) are re-oxidised (NAD+, NADP+, FAD+) through dehydrogenation reactions releasing hydrogen  $(H_2)$  in the rumen. Once produced,  $H_2$ is used by methanogenic archaea to reduce  $CO_2$  into  $CH_4$  ( $CO_2 + 4 H_2 \rightarrow CH_4 + 2$ ) H2O) (Czerkawski, 2013). The effectiveness of the fermentation relies on the removal of  $H_2$  which is primarily completed through methanogenesis (Moss et al., 2000), but it is not the only natural  $H_2$  sink in the rumen. The metabolic pathways for the production of propionate and valerate also serve as  $H_2$  sinks with a net uptake of  $H_2$ , whereas the production of acetate and butyrate results in a net release of  $H_2$ (Van Soest, 1994). Dietary manipulation is a key factor to mitigate  $eCH<sub>4</sub>$  since feed intake (quantity and nutritional composition) influences the quantity and profile of VFA produced in the rumen, and therefore modulates the availability of  $H_2$  necessary in the formation of CH<sub>4</sub> (Molano & Clark, 2008). It was established that CH<sub>4</sub> production can be calculated from stoichiometry of the main VFA formed during rumen fermentation (Demeyer & Fievez, 2000).

#### 1.4.3 Mitigating enteric methane emissions

Methane is the main GHG emission at the farm level (Veysset et al., 2010). Additionally, methane constitutes an energetic "loss" for the ruminant, ranging from 2 to 12% of its gross energy intake (Johnson & Johnson, 1995). Decreasing  $eCH_4$ from ruminants without altering animal production is both a means of improving feed conversion efficiency and a strategy to reduce the negative environmental impacts of ruminants.

Enteric CH<sup>4</sup> emissions from ruminants can be reduced through various strategies (nutrition, biotechnologies, management, and genetics) but there are few costeffective solutions currently available to producers (Beauchemin et al., 2022). The sustainability of any approach must consider both the effectiveness of  $eCH<sub>4</sub>$ mitigation, and its impact on animals' production performance including health. The effectiveness of mitigation strategies should be evaluated through different metrics: in terms of animal productivity, with the focus on reducing CH4I, per kilogram of product (milk or weight gain) or assessing CH<sup>4</sup> yield (CH4Y), the feed-use efficiency by reducing eCH<sup>4</sup> per kilogram of DMI. The strategies must also consider practical, financial, regulatory, and societal considerations that are key for implementation by farmers. Policy makers must also consider any potential trade-offs that could lead to increased GHG emissions elsewhere in the livestock production system such as in the manure or through importation of feed.

A comprehensive life cycle assessment (LCA) is essential to accurately evaluate the carbon footprint of a system. This allows one to investigate both emissions and sinks (net emissions) of the different GHG ( $CO<sub>2</sub>$ ,  $N<sub>2</sub>O$ ,  $CH<sub>4</sub>$ ) and their sources. Indeed, certain eCH<sup>4</sup> mitigating strategies could have drawbacks in other GHG emissions elsewhere in the livestock system. According to the FAO (2017), the production, processing, and transportation of feeds account for up to 42% of total GHG. It is difficult to assess a system and provide reliable recommendation by looking solely at the emissions from one factor  $(eCH<sub>4</sub>)$ , without considering the rest of the emissions (e.g., feed, mechanisation, transport) and the potential uptake of emissions. The reduction of the emission in one place should not be carried by another nation and should not come at the price of increased eutrophication, acidification, land use, and non-renewable energy use. O'Brien et al. (2012) have shown that seasonal grass-based systems have a lower GHG emissions impact than confinement systems when considering off-farm emissions. A more recent LCA (Sorley et al., 2024) has highlighted that dairy cows in housed farms (indoor feeding) had higher carbon footprints than mixed and grazing farms. They also highlighted that farm management practices explained up to 79% of the variation in carbon footprints. This large variability is a positive finding as it represents a source of potential mitigations.

There is a vast array of literature reviews regarding eCH<sub>4</sub> mitigation strategies currently available (Beauchemin et al., 2022; Boadi et al., 2004; Knapp et al., 2014; Martin et al., 2010). According to Hristov (2024), searching on Web of Science with the terms "methane," "emissions," and "livestock," will retrieve a total of 495 articles in the last 5 years alone, including 84 reviews. A recent meta-analysis conducted by Arndt et al. (2022) highlighted that the most effective mitigation strategies can be classified into three main categories and are applicable in different production systems (feedlot, mixed feeding, and grassland systems): 1) animal and feed management: feed processing, genetic selection, improving animal health and pasture management, increasing feeding level and forage quality, and TMR feeding; 2) diet formulation: by-products, decreasing forage-concentrate ratios, minerals and salts, oils, fats, oilseeds, tanniferous forages, urea, and increasing protein; 3) rumen manipulation: additives, defaunation, and electron sinks.

The first panel of Figure 5 (Arndt et al., 2022) highlights the three most effective product-based strategies (CH4I), while the second panel identifies the most effective strategies for reducing absolute eCH<sub>4</sub>. The three performance-based strategies (milk) resulted in an average 12% reduction in CH<sub>4</sub>I (ranging from 9% to 17%) and a median 17% increase in animal production (from 9% to 162%). In contrast, the five absolute eCH<sub>4</sub> strategies achieved an average 17% reduction in CH<sub>4</sub>I (12% to 32%) and a 21% decrease in absolute  $eCH_4$  (12% to 35%). These two panels illustrate that not all mitigation strategies are suitable for grassland systems. The primary options for milk include two product-based strategies, increasing feed levels  $(-17\% \text{ CH}_{4})$ and reducing grass maturity  $(-13\% \text{ CH}_4\text{I})$ , and one strategy for reducing absolute eCH<sub>4</sub> by using tanniferous forages  $(-12\% \text{ eCH}_4; -18\% \text{ CH}_4)$ . Since most Scandinavian dairy systems utilise mixed feeding, a broader range of mitigation strategies is applicable. As concluded by Arndt et al. (2022), the full-scale implementation of only one product-based or absolute strategy is insufficient to significantly reduce global eCH<sub>4</sub> from agriculture by 2030 or 2050 to meet the  $1.5^{\circ}$ C climate target. However, the simultaneous 100% adoption of the most effective product-based and absolute strategies could reduce global eCH4 enough to meet the 1.5°C target by 2030. Although ambitious and theoretical, this objective emphasises the potential of ready-to-implement eCH<sup>4</sup> mitigation strategies. These could significantly contribute to transforming the dairy sector into a more climate-friendly industry.

A					
	<b>MITIGATION STRATEGY</b>		<b>POTENTIAL EMISSIONS REDUCTION</b>		<b>RELEVANT PRODUCTION SYSTEM</b>
	ſП <b>INCREASING FEEDING LEVEL</b>	<b>CH4IM</b> CH4IG	$-17%$ <b>No Data</b>		
Product-Based Reductions	2 <b>DECREASING GRASS MATURITY</b>	<b>CH4IM</b> CHalg	$-13%$ <b>No Data</b>		
	DECREASING DIETARY FORAGE-TO- CONCENTRATE RATIO ℮	<b>CH4IM</b> CH <sub>4</sub> I <sub>G</sub>	$-9%$ $-9%$		
	п <b>CHAINHIBITORS</b>	<b>CH4IM</b> $-32%$ <b>No Data</b> CH4 <sub>IG</sub>	Daily CH <sub>4</sub> $-35%$ <b>CHAY</b> $-34%$		
<b>Absolute Reductions</b>	2 <b>TANNIFEROUS FORAGES</b>	<b>CH4IM</b> $-18%$ <b>No Data</b> CH4lo	Daily CH <sub>4</sub> $-12%$ $-10%$ CH <sub>4</sub> Y		
	8 <b>ELECTRON SINKS</b>	$-13%$ <b>CH4IM</b> <b>CH4IG</b> $-12%$	Daily CH <sub>4</sub> $-17%$ <b>CH<sub>4</sub>Y</b> $-15%$		
	4 <b>OILS &amp; FATS</b>	$-12%$ <b>CH4IM</b> CHala $-22%$	Daily CH <sub>4</sub> $-19%$ $-15%$ CH <sub>4</sub> Y		
	<b>OILSEEDS</b> Б ing animals only	<b>CH4IM</b> $-12%$ <b>No Effect</b> CH4IG	Daily CH <sub>4</sub> $-20%$ CH <sub>4</sub> Y $-14%$		
	<b>Production system</b>		FEEDLOT & MIXED SYSTEMS		<b>GRASSLAND SYSTEMS</b>
B					
			<b>Relative Treatment Effect on Animal Performance</b>		
	<b>MITIGATION STRATEGY</b>	<b>INTAKE</b>	DIGESTIBILITY	<b>MILK</b>	GAIN
	П <b>INCREASING FEEDING LEVEL</b>	+58%	$-7%$	$+17%$	+162%
	Р <b>DECREASING GRASS MATURITY</b>	No Effect	$+15%$	$+9%$	<b>No Data</b>
Product-Based Reductions	DECREASING DIETARY FORAGE-TO- Concentrate Ratio €	+9%	<b>No Effect</b>	$+17%$	$+21%$
	$\bm\Omega$ <b>CH, INHIBITORS</b>	No Effect	No Effect	No Effect	<b>No Effect</b>
	<b>TANNIFEROUS FORAGES</b> P	No Effect	$-7%$	<b>No Effect</b>	No Effect
	8 <b>ELECTRON SINKS</b>	$-2%$	<b>No Effect</b>	$+3%$	<b>No Effect</b>
<b>Absolute Reductions</b>	<b>OILS &amp; FATS</b> G.	$-6%$	$-4%$	No Effect	<b>No Effect</b>

Figure 5. Effective mitigation strategies and their effect on enteric methane (eCH4) emissions (A) and animal performance metrics (B). CH<sub>4</sub>IM = CH<sub>4</sub> emission intensity for milk (g CH<sub>4</sub> kg of milk); CH<sub>4</sub>IG = CH<sub>4</sub> emission intensity for weight gain (g CH<sub>4</sub> kg of weight gain for growing/animals); daily  $CH_4$  = daily  $CH_4$ emissions( $g/$ animal/d); digestibility = apparent digestibility of neutral detergent fibre (%); gain = average daily gain (kg/d); intake = dry matter intake (kg/d); milk = milk yield (kg/d); when numeric values are shown a significant effect was observed (adjusted  $p < 0.05$ ) and no effect when adjusted  $p \ge 0.05$  (Source: Arndt et al., 2022).



Figure 6. Map of the location of each experiment in Papers **I**-**IV** in Norway and Sweden with the journal name and the status of the paper (published or submitted), and the date of trial and publication.

# 2. Objectives and hypothesis

### 2.1.1 Objectives

This aim of this thesis was to evaluate different dairy cows' grazing management in Nordic conditions. The evaluation focused on dairy cows' performances (intake, milk production; Papers **I**, **III**, and **IV**), the behaviour response of animals (Papers **I**, **III**, **IV**) and pollutant waste from animals, specifically exploring their  $eCH_4$ (Papers **I** and **IV**). The applied objective of this work was to identify simple feeding and grazing management changes that could optimise farmers' production systems. The Paper **II** was a bit of a sideline to the thesis, but it's still interesting because it allowed us to quickly explore the relationship between eCH<sup>4</sup> and feed quality.

### 2.1.2 Hypothesis of each paper

The general hypothesis of this thesis was that the inclusion of a significant amount of grazed grass in the diet of dairy cows during summer would maintain animal performances. The following hypothesis was that the replacement of grass silage by grazed grass in their diet would reduce the overall eCH<sup>4</sup> of dairy cows.

Each paper has been given a concise running title summarising the primary treatment or hypothesis explored in the study. Before delving into the materials and methods of each paper, the scientific hypotheses of each paper are outlined below:

#### I. Paper **I**: Day or night-time grazing

The hypothesis for this paper was that night-time grazing in a PTG system, rather than daytime grazing, will increase herbage intake on pasture and milk performance by aligning with cows' natural diurnal behaviour patterns, and thereby reducing eCH<sub>4</sub>Y and eCH<sub>4</sub>I. The hypothesis was that daily eCH<sub>4</sub> recorded outdoors (fed fresh grass) and indoors (fed PMR) would differ.

## II. Paper **II**: Herbage quality and methane emissions

The hypothesis for this study was that enhanced grass quality caused by altered management and weather, would reduce the methanogenic potential of the feed whilst the DMI of sheep would increase.

## III. Paper **III**: Morning or afternoon new grazing strips

The hypothesis of this paper was that providing lactating dairy cows with fresh pasture in the afternoon or early evening, rather than in the morning, would match their motivation to graze, increase intake quantity and/or quality, and so improve milk performance.

## IV. Paper **IV**: Exercise or production pasture access

We hypothesised that: 1) dairy cows that were provided access to production pastures in a PTG system would exhibit lower eCH<sup>4</sup> and similar performances compared to cows with an exercise paddock in an AMS equipped barn; 2) cows managed under AMS with access to production pastures would spend more time outdoors than those provided with recreational exercise paddocks; 3)  $eCH_4$  of dairy cows recorded outdoors (fed fresh forage) would be lower than indoors (fed conserved forage).



Photo: Horacio Gonda

	Paper			≥
	Short running title	Day or night pasture access	Morning or afternoon new strip	Excercise or production pasture access
General	<b>Status</b>	Published: Acta Scandinavia, Section	Published: Journal of Applied Animal	Submitted: Journal of Dairy Science
information	Location	Sweden, Umea	Koinge, Sweden	Norway, Maere
	Infrastrucutre	SLU experimental dairy barn	Commercial organic dairy barn	Landbrukskoole dairy barn
	Treatment (abreviation)	DAY & NIGHT	AM & PM	EX&PROD
Experimental	Desing of the experiment	Case control study	Case control study	Change over design
treatment &	Adaptation, week			
design	<b>Barn feature</b>	Milking parlour	Milking parlour	AMS barn
	Max pasture distance, m	200	1000	300
	Breed	<b>Red Nordic</b>	<b>Red Nordic</b>	<b>Red Nordic</b>
	Number of animal		S	32
Animal	Days in milk, d	<sup>ន ខ្ល</sup>	154	159
	Body weight, kg	625	616	650
	Parity	$\overline{u}$	$\frac{6}{16}$	22
	Feeding strategy	Mixed feeding	Full time grazing	Mixed feeding
	Feeding regime	PTG with ad lib PMR	Full grazing with grain	PTG (restricted grass silage + grazing) or
Feeding			supplementation	ad lib grass sialge
	Herbage contribution in DMI, %	$-20$	8	$-0$ or 50
	Level of concentrate, %	<del>q</del>	$\overline{20}$	Individually adpated
	Pasture type	Temporary pasture	Temporary pasture	Temporary pasture
Pasture	Grazing management	<b>Stripgrazing</b>	Stripgrazing	Strip forward grazing
	Allowance, kg DM/cow/d	g	$\frac{40}{7}$	g
	Recording period, d		in,	4
	Pasture measurments	Botanical, height, mass & availabilty	Botanical, height, mass & availabilty	Botanical, height, mass & availabilty
	Methane measurement	GEM: indoor and outdoor		GEM: indoor and outdoor
	ntake measurement	Recorded indoor & estimated outdoor Recorded indoor & estimated outdoor		Recorded indoor & estimated outdoor
Recording	Feed quality analysis	NIRS	NIRS + chemical analysis	NIRS
	<b>Milk</b> yield	Automated recording	Manual recording	Automated recording
	Milk quality	Mid-infrared spectroscopy	Mid-infrared spectroscopy	Mid-infrared spectroscopy
	Animal behaviour	NEDAP	<b>NEDAP</b>	RTLS (indoor & outdoor)
	Other animal recording	≧	MB	BW, BCS
<b>Statistical</b> analysis	Model	Mixed effect model	Mixed effect model	Mixed effect model

Table 2. Resume of the materials and methods of the three papers on dairy cows (**I**, **III**, and **IV**).

# 3. Materials and Methods

This section presents an overview of the materials and methods used in the four papers. Each paper is presented one at a time. The papers are arranged in numerical order (**I** to **IV**), reflecting their order of publication during the PhD.

A comprehensive summary of the key information from the three papers that form the core of this PhD research (Papers **I**, **III**, and **IV**) are presented in Table 2. This includes essential details related to the general information, experimental treatment and design, animal and pasture characteristics, animal feeding, recording procedures for the different measurements and statistical model. The layout of Table 2 allows for straightforward comparisons between the papers, providing a concise yet thorough snapshot of each study. The rest of the materials and methods can be found in each paper as a compilation at the end of the thesis. The illustration on the opposite page of each article is a simplification of the treatment of each article with certain characteristics of each experimental site.



Day or night-time grazing illustration (Paper **I**, Umeå) Original illustration from Olivier Martin

## 3.1 Paper **I**: Day or night-time grazing

The first paper was a grazing experiment on the effects of day or night-time grazing in a part-time grazing system on milk production, animal behaviour, and eCH<sup>4</sup> emissions.

This grazing experiment was conducted at the Röbäcksdalen research farm at the Swedish University of Agricultural Sciences (SLU) in Umeå, from June 1<sup>st</sup> to July 2<sup>nd</sup>, 2021. The experiment lasted 31 days, with a 24-day (01-25/06) period of adaptation to feed, management routines, and training to the GreenFeed emissions monitoring (GEM) units, followed by seven days of recording (26/06-02/07). The experiment used 30 Nordic Red dairy cows, blocked according to days in milk (DIM), MY, and parity and allocated to one of two grazing treatments: daytime grazing (DAY) and night-time grazing (NIGHT). The DAY group grazed for 10h during the day (0700-1700 h), and the NIGHT group for 12h during the evening and night (1700-0500 h). The rotation, between indoor or pasture access time, was scheduled around milking. All animals received an *ad libitum* partial mixed ration (PMR; 50% silage, 49% concentrate, and 1% mineral) indoors and a high grass allocation (18 kg DM per cow per day) with daily fresh strips from a grass-clover pasture. This grass allocation was based on a daily intake of 6 kg DM from previous years multiplied by three to ensure high allowance and no competition over feed. Silage, concentrate, and pasture quality were all monitored during the recording period. Grass DMI was estimated by subtracting recorded intakes (PMR intake and concentrate intake) from the estimated total DMI (De Souza et al., 2019). Methane emissions were measured using two GEM units, a mobile unit located in the pasture and a fixed unit in the barn. The outdoor GEM was moved twice a day to the new offered strip (once for DAY and once for NIGHT). These units were wirelessly linked and considered as a single unit in the calculation of  $CH<sub>4</sub>$  emissions from individual animals. All animals were equipped with Nedap SmartTag Neck sensors, which automatically recorded four different behavioural states (eating/grazing, ruminating, resting, and other).

Data was subjected to an ANOVA using a mixed effect model (R Core Team, 2021) to test the effects of the two grazing treatments (DAY, NIGHT), with DIM, parity and pre-experimental MY as covariates (except for behaviour data). A secondary mixed effect model was used to investigate the daily eCH<sup>4</sup> emissions per animal and per GEM unit. Cow was used as a random factor and the environment of the GEM units, indoor or outdoor, were used as a fixed factor. This second model was employed to compare CH<sup>4</sup> emissions recorded indoors and outdoors.



Herbage quality and methane production (Paper **II**, Tjøtta) Original illustration from Olivier Martin

## 3.2 Paper **II**: Herbage quality or methane production

The second paper was partly an *in vivo* and *in vitro* experiment with a modelling approach to estimate CH<sup>4</sup> production, digestibility, and rumen fermentation patterns based on two different qualities of Italian ryegrass (*Lolium multiflorum*) pasture grazed by sheep (non-pregnant ewes).

*In vivo*: Sheep grazing and herbages collection of pastures were carried out at the Tjøtta experimental station (NIBIO) in Norway during the summer of 2020. Herbage sampling and grazing of the paddocks was carried out in two 10-day recording periods, July (16-25/07; low-quality pasture) and August (11-20/08; high-quality pasture). The unfertilised pasture was cut 10 days prior to the first period. Prior to period two, the pasture was mowed, fertilised with 20 kg/ha of mineral fertiliser (12- 4-18 NPK) and left to grow for 30 days. The sheep were adapted during the month prior to period one and two weeks prior to period two on an adjacent pasture. The pasture was delimitated into four blocks, and the 16 non-pregnant ewes were grouped (initial live weight and age) and allocated to one block over the recording period. Each block had five grazing strips of two days. The pasture measurement was performed following the same routine, prior to the ewes' entry to their new strip. Herbage height and samples were collected using a quadrant, a plate metre, and mechanical clipping before grazing. Herbage height was also measured after the subplot was grazed. The DMI was estimated using the herbage disappearance method.

*In vitro*: Herbage samples were dried in an oven and milled, the grass samples from each strip were incubated in a dairy cow's rumen fluid with a fully automated *in vitro* system as described by Cone et al. (1996) in SLU Umeå. The concentration of CH<sup>4</sup> was determined by sampling the gas in the headspace at multiple intervals during incubation: 2, 4, 8, 24, 32, and 48 hours. It was then introduced into a Varian Star 3400 CX FID Gas Chromatograph (Varian Inc., Palo Alto, CA) for analysis. Upon reaching the 48-hour incubation time, liquid samples were extracted to evaluate the concentration of VFA by utilising ultra-performance liquid chromatography, as per the methodology outlined by Puhakka et al. (2016). We also performed *in vitro* incubations to determine organic matter digestibility (OMD) of the herbage samples. Data on total gas and CH<sup>4</sup> production parameters, total VFA production, and molar proportions of VFA were subjected to statistical analysis by a mixed effect (R Core Team, 2021), with the sampling period (July and August) as a fixed effect and bottle, run, and days as random effects. The chemical composition of herbages was analysed as the mean per subplot using the same model without run and bottles.



Morning or afternoon new strip (Paper **III**, Koïnge) Original illustration from Olivier Martin

## 3.3 Paper **III**: Morning or afternoon new strip

The third paper was a grazing experiment investigating the effect of time of fresh pasture's allocation on milk production and behaviour of dairy cows in a full-time grazing system.

This study was conducted at a commercial organic dairy barn in the Hallandcounty, from May  $5<sup>th</sup>$  to May  $28<sup>th</sup>$ , 2022. The first two weeks were used to allow the cows to adapt to the new groups and to the grazing management system, and the last five days were used for data collection and sampling. Sixty dairy cows were allocated based on DIM, MY, and parity, into two treatment groups: new strip in the morning (AM; n=30) and new strip in the afternoon (PM; n=30). Pre-experimental recordings were collected and analysed to ensure no-significant differences in average MY and time spent grazing before adaptation. The cows were offered strip grazing with herbage allowance (>40 kg DM/cow/d) after either morning milking or afternoon milking. Cows were milked twice daily (0500 and 1500 h) and received 2 kg grain-mix per milking (in total 4 kg DM/cow/d). Cows grazed on several pasture plots, following the farmer's normal routine, in a daily strip grazing system using temporary electric fencing. The pasture used was established in 2021 using a seed mixture comprising 30% perennial ryegrass, 26% timothy, 17% meadow fescue, 13% white clover, 6% chicory, 4% plantain and 4% cumin.

Each cow's daily DMI was estimated using the equation described by De Souza et al. (2019). The cows' behaviour was automatically recorded using Nedap SmartTag neck sensors (54 cows) which automatically recorded four different behavioural states (grazing, ruminating, resting, and other). Before the cows had access to a new grazing strip, the botanical composition of the pasture was determined using the dry-weight-rank method (Mannetje & Haydock, 1963). Simultaneously, the herbage samples of pasture were hand-picked at 30 sites per day per treatments, pooled and dried, and analysed by conventional chemical analyses. Herbage mass was measured daily using a rising plate metre immediately before the cows accessed their new strip. Milk yields were recorded at each milking during the sampling period, and samples for milk composition were collected during the last four milkings of the sampling period.

Statistics were computed on the sampling period mean per cow for productive variables and behaviour. The effects of the treatments on behaviour, feed intake, body weight change, milk yield, and milk composition were analysed in a general linear mixed model (SAS 9.4 2016; Cary, NC, USA). Variables included in the model as fixed effects were treatment (AM and PM), parity (primiparous and multiparous), DIM (continuous variable), and the interaction of treatment x parity. Pre-experimental MY was used as a covariate in the analyses for MY.



Exercise or production pasture access (Paper **IV**, Mære) Original illustration from Olivier Martin

## 3.4 Paper **IV**: Exercise or production pasture access

The fourth paper was a grazing study investigating the effect of two contrasting uses of pasture, production or exercise pasture, in a PTG system on milk production and eCH4 emission of dairy cows in an AMS farm.

This grazing study was conducted on the dairy farm of Mære Agricultural School in Trøndelag, from June  $12<sup>th</sup>$  to August  $7<sup>th</sup>$ , 2023. The experimental design was a changeover design with two recording periods, 26/06-09/07 (R1) and 24/07-07/08 (R2). The cows were adapted to their treatment for 2 weeks prior to the two weeks of recordings. The 32 Norwegian Red dairy cows were allocated to two groups based on DIM, MY, BW, ECM and exposed to each treatment, production pasture (PROD), and exercise pasture (EX) on one of the recording periods. The PROD treatment provided cows with access to a temporary pasture for grazing and a restricted amount of silage indoors (6 kg DM/cow/d). EX cows had access to an exercise area with highly limited herbage availability and unlimited indoor grass silage. Concentrate allowance was individually adapted and adjusted throughout the study according to a standardised lactation curve. It was delivered at the AMS, concentrate feeder, and GEM units. The pasture used for the PROD was the same for the other two recording periods and consisted of newly sowed perennial pasture fertilised before each recording period. The grazing management was daily strip grazing with a daily herbage allowance of 18 kg DM per day per cow (2 times the expected intake). The strip grazing had no back fence and unused biomass was available for the next day. Data on temperature, humidity, and rainfall was collected using weather stations (indoor and outdoor). Pasture characteristics, herbage, and silage samples were collected regularly for an analysis of feed quality by nearinfrared spectroscopy (NIRS). Milk yield, BW, and eCH<sup>4</sup> emissions (indoor and outdoor) were automatically recorded for each cow. Only the cows in the PROD treatment were monitored for CH<sup>4</sup> emissions on pasture, whilst both groups were recorded indoors. The outdoor GEM was located at the entry to the pasture. The position (indoor or outdoor) of the cows were calculated based on the outdoor selection gate tag (out) and any over tag recorded indoor (in). Herbage DMI was back calculated using the NorFor equation (Volden, 2011) by subtracting the total net energy (NE) intake recorded by the total NE requirement for each cow per day.

Two mixed-effect ANOVA models were used in the statistical analysis (R Core Team, 2021). The first model assessed total DMI, milk production, eCH<sub>4</sub> emissions, BW, and behaviour. It included treatment (EX or PROD), period (R1 or R2) as a fixed factor, cow as a random effect, and covariates for days in milk and lactation stage. The second model focused on comparing eCH<sub>4</sub> emissions between indoor and outdoor GEM units for the PROD treatment, considering GEM location and period as fixed factors, with cow as a random factor.

	Paper						≥
	Short running title	Day or night pasture access		Morning or afternoon new strip			Excercise or production pasture access
	Treamtent	<b>DAY</b>	NIGHT	ξ	看	ă	PROD
	Silage quality, NE MJ/kg DM	$\overline{6.0}$					6.0
Feed	Concentrate quality, NE MJ/kg DM	$7.3 - 7.4$		$7.2^{3}$			$6.9 - 7.4$
	Botanical composition, % species	[(37%), WC (29%), MF (21%)		30% PR, 26% T, 17% MF, 13% WC			65% T, 20% MF, 5% RC
	Pasture quality, NE MJ/kg DM	6.4	6.4	23	7.3	low <sup>e</sup>	$^{1.4}$
Pasture	Pre-grazing sward height, cm	$\overline{20}$	$\overline{20}$	25	26		$18 - 24$
	Herbage mass, kg DM/ha	2684	2735	2802	2918	$\sum_{i=1}^{n}$	2300
	DMI, kg DM	21.6	$\overline{20.1}$	20.1	20.4	16.8	16.6
	NE intake, MI/d	133	136	128	137	119	113
Intake	Estimated pasture intake. kg DM	4.9	4.5	16.1	16.4	$0.6^\circ$	$\frac{5}{8}$
	Concentrate intake, kg DM	o,	$\frac{4}{3}$			5.7	9.9
	Silage intake, kg DM	7.6	7.3		O	10.4	4.2
	Milk yield, kg M)	26.3	26.0	25.8	27.7	23.3	22.6
Production	ECM, kg ECM	29	28.2	26	28.6	24.3	23.8
metrics	BW change, kg	0.1	0.2	$-0.7$	$-0.6$	$-0.7$	0.6
	eCH4,gCH4	373	370	. 334	. ೫೫	387	326
	eCH4 Indoor, g CH4	399	426			387	359
	eCH4 Outdoor, g CH4	285	301				300
Methane	CH4Y, gCH4/kg DM	17.3	18.3	$16.6^{\circ}$	$16.5^\circ$	24.5	19.7
	CH41, 8 CH4/K8 ECM	13.4	13.4	12.8 <sup>d</sup>	$11.8^d$	18.5	13.4
	Visit, number/d	$\frac{9}{3}$	3.3			H	0.7
	ime spent grazing, min	44	462	520	576	$252^e$	660°
Behaviour	Time spent ruminating, min	468	456	468	409		
	Time spent idling, min	486	492	359	330		
	Abbreviations: WC: White clover; T: Timothy; MF: Meadow fescue; PR: Perennial ryegrass; RC: Red clover						
	NE value calcualted based on the composition of the grain mixed (50% barley, 25% wheat, 15% rye, 10% oats) and the energy value from NorFor (Volden, 2011)						
	unmanaged paddock with grass cover						
	estimated intake based on NE requirement and NE intake not matching (0.6 kg) that was assumed to be grazed if NE deficinecy for the cows in EX treatment						
	calculated for the thesis with the equation from Niu et al. (2018): CH $_4$ = 26.0 + 15.3 × DMI + 3.42 × NDF/10						

Table 3. Resume of the results of the three papers on dairy cows (**I**, **III**, and **IV**).

" only represent time spent outdoors

# 4. Results

The following section provides a detailed overview of the results derived from the four papers included in this thesis. Each paper's results are presented individually and in numerical order (**I** to **IV**). On the opposite page of each paper results are displayed in a table or as a figure of interest.

A comprehensive summary of key findings from the three primary papers of this research PhD (**I**, **III**, and **IV**) is presented in Table 3. Results have been expressed in common units in this table to compare data across experiments and to facilitate the discussion. In addition, eCH4 emissions (Paper **III**) have been estimated using the equation provided by Niu et al. (2018). Other detailed results can be found in each paper, presented as a compilation at the end of the thesis.



Figure 7. Diurnal pattern of methane (CH4) emissions, eating behaviour, and visits to a GEM unit for daytime (DAY) pasture access (0700-1700 h) and night-time (NIGHT) pasture access (1700-0500 h). (A and C) Mean enteric CH<sub>4</sub> emissions (g/h) per hour, where horizontal lines indicate mean  $CH_4$  recorded by each unit (upper line = indoor GEM, lower line = outdoor GEM), and the vertical dashed line at 0230 h represents sunrise and the vertical dashed line at 2300 h represents sunset. (B and C) Mean eating time per hour (min) recorded with the NEDAP system, where bars represent the sum of visits per hour to the accessible GEM unit at that time of day (filled for indoor, crosshatched for outdoor). The lines  $(CH<sub>4</sub>$  and eating behaviour) in all panels are identical, showing the location of the animals at a given time of day (dotted line whilst cows were indoors, dotted line transition during milking, solid line whilst cows were outdoors).

# 4.1 Paper **I**: Day or night-time grazing

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. The herbage had a 42% lower DM content compared to the PMR, but similar energy (11.3 vs. 11.8 MJ ME/kg DM) and CP contents (186 g/kg DM). The NDF content was higher in the herbage than in the PMR due to the high level of concentrate. Silage had 32 g NDF/kg DM higher than herbages. Chemical composition of herbage across treatment strips was numerically similar, and pasture characteristics were consistent between treatments. The pasture characteristics were numerically identical across treatments, pre- and post-grazing compressed height, indicating uniform grazing conditions between treatments.

Animal performance metrics showed no significant difference between the groups in intake of the PMR ( $p = 0.317$ ) and estimated herbage intake ( $p = 0.575$ ). However, intake of concentrate was significantly higher for the DAY group (9.0 and 8.3 kg DM/d for DAY and NIGHT, respectively;  $p = 0.006$ ). Estimated total DMI was higher for cows in the DAY group (21.7 kg DM) than in the NIGHT group (20.2 kg DM) ( $p = 0.012$ ). Milk yield, ECM, and milk composition did not statistically differ between the two treatments ( $p > 0.05$ ).

Methane emissions showed no significant effect of the treatments on absolute CH<sub>4</sub> emissions (373 and 370 g CH<sub>4</sub>/d for DAY and NIGHT respectively;  $p > 0.05$ ). However, a significant difference was found in eCH<sub>4</sub> recorded between the indoor (414 g CH<sub>4</sub>/d) and outdoor (300 g CH<sub>4</sub>/d) GEM units ( $p < 0.0001$ ). The differences in CH<sup>4</sup> emissions between indoor and outdoor measurements are displayed in Figure 1(A) (DAY) and Figure 2(A) (NIGHT). As these diagrams show, 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). In terms of GEM unit visits, cows in the DAY group visited the units 3.9 times per day, comprising 75% of their visits to the indoor GEM. In contrast, cows in the NIGHT group visited the units 3.3 times per day, equally distributed between the indoor and outdoor units.

For animal behaviour, NIGHT cows spent more time grazing  $(0.8 \text{ h more, p} <$ 0.001) than DAY cows. However, time dedicated to grazing as a percentage of access time to pasture was higher for the DAY group ( $p = 0.002$ ). The NIGHT cows had a higher grazing activity during the first two hours on pasture compared to DAY cows ( $p < 0.004$ ).



Abbreviations: CH<sub>4</sub>, methane; DM, dry matter; SEM, standard error of the mean.

Table 4. Effect of harvesting herbage in July or August on predicted *in vivo* total gas and CH<sup>4</sup> production.



Abbreviations: A, acetate; DM, dry matter; P, propionate; SEM, standard error of the mean; VFA, volatile fatty acid.

Table 5. Effects of harvesting herbage in July and August on *in vitro* total VFA production, VFA molar proportions, and VFA molar ratio at 48 h of incubation of buffered rumen fluid.

## 4.2 Paper **II**: Herbage quality and methane production

The sheep DMI was significantly higher in August (2.4 kg DM/d) compared to July  $(1.5 \text{ kg } DM/d)$  ( $p < 0.005$ ). The chemical composition of herbage significantly differed for all parameters between the two periods, except for potentially digestible NDF ( $p = 0.07$ ) and water-soluble carbohydrates (WSC;  $p = 0.23$ ). The WSC:CP ratio in July (2.03) was twice that in August (1.02). August herbage had a higher ME content ( $p < 0.005$ ) and CP concentration (+32%) compared to July. Sward height before and after grazing was greater in August (27.5 and 8.8 cm, respectively) than in July (13.5 and 5.8 cm, respectively). However, the herbage mass offered to the animals was similar due to the lower DM concentration of the grass in August (15%) compared to July (20%) ( $p > 0.05$ ).

*In vitro* gas production and fermentation rate (Table 4) in July were significantly higher ( $p \le 0.005$ ) compared to August (+8% and +19%, respectively). Asymptotic CH<sup>4</sup> production and predicted *in vivo* CH<sup>4</sup> production were higher for July herbage compared to August ( $p < 0.005$ ). No significant difference (Table 5) was found in total VFA and molar proportion of butyrate between periods ( $p = 0.458$  and 0.273, respectively). However, there was a significant difference in the A:P ratio in the incubations of the herbage from July ( $p = 0.029$ ), with August having a lower ratio in the buffered rumen fluid than the herbage from July.



Figure 8. Diurnal behaviour pattern of lactating dairy cows as the percentage of minutes spent per activity in the treatments. (A) access to new pasture after morning milking (AM) and (B) access to new pasture after afternoon milking (PM).



Figure 9. Mean, standard error, F-value, and statistical significance (\*<0.05 and \*\*\*<0.001) of difference in minutes spent eating during grazing in the 2, 4, and 6 hours after morning and evening milking for lactating dairy cows receiving new pasture after morning milking (AM) or after afternoon milking (PM).

## 4.3 Paper **III**: Morning or afternoon new strip

The herbage characteristics of the pastures and chemical composition were similar for the AM and PM treatments with mean metabolisable energy contents of 12.1 and 12.3 MJ/kg DM, respectively. Herbage mass, pre- and post-grazing surface sward height, and herbage allowance were almost identical for both treatments. However, the CP:WSC ratio was numerically higher in AM grass, lower in AM clover, and chicory proportions were also higher in the AM treatment.

The milk yields were unaffected by the allocation time of a new strip, but cows in the PM group had higher ECM ( $p < 0.01$ ; 20.1 and 20.4 kg, respectively) and milk protein yield ( $p < 0.05$ ; 26.0 and 28.6 kg, respectively) than cows in the AM group. Milk fat and lactose production per day were typically higher in the PM group, but concentration of milk urea nitrogen was generally lower in the PM group. There was an effect of DIM on all variables except estimated DMI (20.1 and 20.4 kg DM, respectively), milk protein yield per day, and urea in milk. No differences were found in estimated pasture intake and body weight between the treatments.

Regarding behaviour, the PM group grazed for a longer duration ( $p < 0.001$ ; 520 and 576 min, AM and PM respectively) and ruminated for a shorter duration ( $p <$ 0.001; 468 and 409 min AM and PM, respectively) than the AM group. Grazing and rumination both showed activity peaks after milking and at dusk (Figure 8), and the cows switched from rumination to grazing around dawn. The PM group grazed more around dusk, whilst the AM group's grazing was evenly distributed throughout the day. There was a significant difference in grazing time (min/h) between the two treatments for each time interval studied (2, 4, and 6 h; Figure 9), with the cows that received a fresh strip spending more time grazing than the cows grazing an older strip. There was a cumulative numerical increased effect for the PM cows, who after 6 h on fresh pasture had spent 228 min grazing, compared with the AM cows, who spent only 146 min out of 6 h grazing on fresh pasture.



Figure 10. Diurnal pattern of the average time spent outdoors (A), methane emissions (B), milk yield (C), and silage intake (D) per hour for the PROD treatment (blue) and for EX treatment (red). The panels A, C, and D display the average time spent outdoor (h/h), milk yield (kg MY/h) and silage intake (kg DM/h) (line), and the number of visits (number/h) to the recording device for each measurement (bar plot) per hour per cow per treatment, respectively. Panel B displays the average CH<sub>4</sub> emissions (g CH<sub>4</sub>/h) and the total number of visits per GEM units per hour per treatment.

## 4.4 Paper **IV**: Exercise or production pasture access

The botanical composition of the pastures was similar across the two periods. The dominant species were timothy (53%), meadow fescue (40%), smooth meadow grass (12%), and unsown legumes and weeds (5%). Pre-grazing sward heights were lower in R1 (18.5 cm) compared to R2 (24.5 cm). Pre-grazing herbage mass averaged 2,194 kg DM/ha in R1 and 2,682 kg in R2. The daily herbage available to cows in the PROD treatment provided an allowance of 22 kg DM/day across both experimental periods.

Feed intake differed between treatments as intended by the experimental design. The cows in the EX treatment consumed 10.4 kg DM/d of silage, while cows in the PROD treatment consumed 4.2 kg DM/d of silage. Cows in the PROD treatment had an estimated herbage intake of 8.5 kg DM/d compared to 0.6 kg for EX cows. The TDMI was similar between the treatments at 16.6 and 16.8 kg DM/day for PROD and EX, respectively, corresponding to net energy intakes of 119 and 113 MJ/day. Forage-to-concentrate ratios were 76% for PROD and 65% for EX, while pastureto-silage ratios were 67% and 5%, respectively.

Cows in the PROD treatment had a lower milking frequency ( $p < 0.001$ ) and lower overall milk yield ( $p = 0.032$ ) compared to cows in the EX treatment. However, the ECM yield was not different ( $p = 0.101$ ) between the two treatments. Period significantly affected all parameters ( $p < 0.001$ ) with cows in R1 having higher milking frequencies, milk yield, and ECM milk compared to R2 ( $p < 0.001$ ). The BW change was not significantly different between the two treatments ( $p =$ 0.098) whereas period had an affect with cows in R1 losing weight but cows in R2 gaining weight ( $p < 0.001$ ). The milk solid composition did not show significant differences for milk fat, milk protein, or SCC between the two treatments ( $p > 0.1$ ). Regarding the effects of the period on milk solids, both milk fat and SCC decreased from R1 to R2 ( $p$ < 0.05) whilst no significant differences were observed in milk protein ( $p = 0.094$ ). The cows in the PROD treatment spent significantly more time outdoors and had a greater number of exit compared to cows in the EX treatment (p  $< 0.001$ ; Figure 10).

Absolute CH<sub>4</sub> emissions were lower (326 and 387 gCH<sub>4</sub>/d;  $p < 0.001$ ), and both CH<sub>4</sub>Y (19.4 and 22.1 g CH<sub>4</sub>/kg DM;  $p < 0.001$ ) and CH<sub>4</sub>I (13.2 and 13.9 gCH<sub>4</sub>/kg ECM;  $p = 0.015$ ) were also reduced in the PROD cows compared to EX cows, respectively. Methane emissions recorded from the outdoor GEM unit in the PROD treatment were significantly lower (297 g CH<sub>4</sub>/d) than those recorded indoors (357 g CH $_4$ /d;  $p < 0.001$ ). Cows visited the indoor GEM unit more frequently than the outdoor GEM ( $p < 0.001$ ).



Figure 11. Visualisation and simplification of the different grazing systems studied through its intensification as the percentage of fresh and conserved forages, and of concentrate in the diet during summer.

# 5. Discussion

Through the compilation of these four articles, this thesis explores different grazing management systems in both Norway and Sweden. The main research papers (Papers **I**, **III**, and **IV**) explore the zootechnical performance in dairy cows (intake and milk quantity and quality), animal behaviour, and  $eCH<sub>4</sub>$  across various grazing management systems that differ in their utilisation of grazed grass, ranging from full to minimal or, in some cases, complete absence of pasture intake. Additionally, it investigates how simple adjustments in grazing practices can improve overall dairy milk performances.

## 5.1 Diversity of feeding management systems studied

The diversity of feeding management systems in relation to the proportion of pasture intake in the diet studied is an asset of this thesis. It explores different existing systems in the sheep sector in Norway and the dairy sector in both countries but displays very different pasture utilisation. These feeding systems are distinguished by varying levels of production intensity (Figure 11) when comparing their respective proportion of transformed feed (concentrate), conserved forage (grass silage), and grazed grass in the animals' diet.

Extensive systems (Papers **II** and **III**) represent full grazing systems with grass as the primary feed source, with no conserved forage in the diet during summer. Grass makes up 100% of the DMI for sheep (Paper **II**) and 76% for dairy cows (Paper **III**). Sheep farming in Norway is extensive, relying on both temporary and permanent pastures for grazing during the summer period. The feeding system involves seasonal shifts in pasture types to optimise feed intake and lamb growth. Sheep obtain approximately 60% of their annual feed intake from grazing (Bhatti et al., 2020). Much of their intake comes from permanent grasslands in the utmark, with minimum daily herd management. For most of April and May they graze temporary infield pastures, transitioning to high-quality permanent pastures in the utmark from June to August, and returning to temporary pastures in September and October (Ross et al., 2016). Concerning the commercial dairy farm (Paper **III**), it was certified organic, following the Swedish regulations for organic dairy production (Swedish Board of Agriculture, 2021), which requires at least 60% of an animal's DMI to be forage. The Swedish Board of Agriculture has requested that during summer a farmer "must plan the feeding of your animals so that you make maximum use of the pasture.". The farmer from Paper **III** was also certified (KRAV), leading an organic label in Sweden mandating that at least 50% of the daily intake of feed calculated as DM must originate from grazing during the grazing period (KRAV, 2021). This farmer exceeded these requirements, maintaining a higher pasture DMI due to his cost-saving strategy, as grass was the most economical feed available (personal communication). The use of transformed feed in his system is minimal with only 4 kg/d/cow of crushed grains produced on the farm. This system is not uncommon for organic dairy barns at this latitude but the production of organic milk in Sweden is quite low (14% of dairy cow's population in 2023) (Swedish Board of Agriculture, 2024a). The significance of grazing in these two systems is also underscored by their seasonal calving strategy, specifically timed for the grazing months.

Intensive systems (Papers **I** and **IV**) are mixed feeding systems with PTG management. These systems are more intensive than the previous one, as indicated by the higher proportion of grass silage and concentrate in the diet (58 to 97%). In Paper **I**, grazed grass served as a supplementary feed in the feeding strategy as it accounted for only 23% of total DMI. The indoor PMR, provided *ad libitum*, contained a relatively high concentrate level (42%). In Paper **IV**, the two treatments incorporated silage and concentrate indoors, but the PROD treatment had a restricted silage allocation. Although the total forage percentages were relatively similar between the two treatments (75% for PROD and 65% for EX), the design of the experiment mechanically reduced the proportion of silage intake in PROD. The cows in the EX treatment were observed grazing, and it was assumed that their individual negative energy balance could have been offset by marginal herbage intake (set at a maximum of 1 kg DM/d). This resulted in a small herbage intake of 3%, or 0.6 kg DM/day. Notably, the proportion of concentrate was also reduced by 10% for the PROD treatment compared to EX treatment. These design adjustments aimed to favour herbage intake by providing grazed grass within the diet of two different feeding management systems.

Rotational grazing management was implemented systematically in all trials with various iterations of strip grazing: daily strip (Papers **I** and **III**), 2-days strip (Paper **II**), and daily forward strip grazing (Paper **IV**). The strip grazing management used in the Papers **I** (DAY) and **III** (AM and PM) were the farmers' routine management. In Paper **IV**, the strip grazing was implemented to maximise pasture intake (Kismul et al., 2018 and 2019), rather than the usual continuous grazing approach of these barns. The information that I was able to gather on the grazing management in both countries suggests that continuous grazing is the most popular management system in Sweden (59%) (Karlsson et al., 2024) for conventional dairy barns. In Sweden, the organic dairy farms preferentially adopted rotational grazing (69%) (Karlsson et al., 2024) as in Paper **III**. In Norway, only Kismul et al. (2020) provided insights, which showed that most farmers (87%) gave pasture access to their dairy herd (without distinguishing between production or exercise pasture) but did not investigate grazing management nor pasture intake. In the absence of online information on grazing management in Norway, one would assume that it is continuous grazing in conventional production, similar to that of Sweden. In the region, dairy and sheep farmers are predominantly cultivating temporary pastures to produce forage (fresh and conserved) for year-round feeding and featuring botanical compositions akin to those found across most high-latitude regions. The botanical compositions were similar between all papers with species commonly used in Nordic temporary pastures, including timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), red and white clover (*Trifolium pratense* and *Trifolium repens*), and perennial ryegrass (*Lolium perenne*) (Ergon et al., 2018). The botanical composition of Paper **III** was more diverse with chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and cumin (*Cuminum cyminum*) believed to increase the resilience of the pastures and provide a more consistent yield throughout the summers, according to the farmer in Paper **III** (personal communication).

How does the pasture intake recorded in our trials compare with information on Swedish and Norwegian milk production on pasture? There is limited available information about the amount or proportion of grazed grass intake in dairy cows' diet in both countries during the grazing season. In Norway, studies (Bakken et al., 2017; Roer et al., 2013; Steinshamn et al., 2021) on grazing dairy cows in different regions of Norway have reported approximately a 10% (ranging between 3 to 17%) contribution of the pasture in the yearly total energy intake. By calculating the monthly contribution of pasture during the grazing months (mid-May to mid-October), it represents approximately 24% of total energy intake, which is quite similar to the results of Paper **I**. However, a Norwegian dairy cow investigation from

Kidane et al. (2018a) performed a grazing experiment with rotational management of different short-term pasture allocation and highlighted that it is possible to rely more heavily on herbage DMI (11.6 to 16.4 kg DM; 73–82% pasture intake proportion on the total DMI), similar to Papers **III** and **IV** (PROD). In Sweden, the report from Reijs et al. (2013) highlights that fresh grass intake was very limited. As stated by Bergsten et al. (2015) and Karlsson et al. (2024) the role of grazing has evolved towards more exercise grazing with most feeding controlled indoors. However, several experiments in Sweden investigating grazing compared to exercise pasture (e.g. Kismul et al. 2018 and 2019) have demonstrated that intake on pasture can be increased (8.7 and 9.0 kg DM/d; 36 and 35% pasture intake proportion on the total DMI) without affecting ECM production.

Despite differences in production and intensification, each of these studied system consistently rely on temporary pasture, either for grazing or production of grass silage, as the primary source of feed during summer. Forages constitute more than 58% of total DMI for all experiments. The information on grazing management and the proportion of herbage intake in the total diet are scarce in both countries. In my opinion, the grazing management system could in many cases be referred to as complementary grazing, definable as pasture intake being inferior to one-third of total DMI. The great diversity of systems and the presence of management with relatively high (> 50% of total DMI) pasture intake indicates the possibility of increasing intake from pasture in the summer diet of dairy cows in Scandinavia.

### 5.1.1 Factors impacting herbage intake on pasture

Dry matter intake is directly related to the energy required for milk production, maintenance, and change in body reserves. It is also affected by the interaction of diet and physiological state (NASEM, 2021). Understanding the factors affecting DMI is essential to optimise the ration formulation and therefore production. Dry matter intake complexity increases in a PTG system wherein animals have access to different dietary options (grass silage, grazed grass, and concentrate), differing in abondance, qualities, and physical characteristics.

The allocation of indoor feed impacts the pasture DMI. A key factor behind this response is the substitution rate, which is the reduction in pasture DMI per kilogram of supplement consumed (Jacobs, 2014). The cows in Paper **I** and **III** had similar total DMI whilst the cows in last study (Paper **IV**) had a much lower intake. Concentrate were fed in all dairy experiments and Paper **I** had the higher concentrate intake (+50%) compared to the two other trials. The grass silage was part of the cows' ration only in Paper's **I** (8.4 kg and 8.0 kg DMI in the DAY and NIGHT) and **IV** (10.4 kg DMI for the EX group and 4.2 kg DM for the PROD). In Paper **I**, the high proportion of concentrate in the *ad libitum* PMR formulation (45%) orientated the cows toward this rich and dense source of feed. Consequently, the pasture DMI was marginal in paper **I** with 4.9 to 4.5 kg DM/cow/d, representing on average 23% of the total DMI in both treatments. When investigating the substitution rate (kg DM of concentrate and silage) and pasture DMI from the three dairy trials, a correlation is found ( $\mathbb{R}^2$ =0.87) towards a pasture DMI reduction of 0.92 kg DM/kg of DM fed indoor. These results are similar to the one reported by Bargo et al. (2003) with a higher substitution rate of 0.84-1.02 kg pasture/kg silage than 0.11-0.5 kg pasture/kg for concentrate (0.7 and 0.2 in our trials for silage and concentrate, respectively). To promote pasture intake in Paper **IV**, we restricted the silage intake indoors, and results were conclusive. The pasture DMI in Paper **IV** (PROD) was doubled compared to the pasture DMI of Paper **I**. The estimated pasture DMI of the cows in the PROD treatment (8.5 kg DM/d) was consistent with the findings of Kismul (Kismul et al., 2019; Kismul et al., 2018), who reported 8.7 and 9.0 kg DMI/d, respectively, in trials with similar pasture access as here. When there was no silage supplementation (Paper  $III$ ), the dairy cows from both treatments achieved relatively high pasture DMI. Based on Van Vuuren and Van den Pol-van Dasselaar (2006), the potential threshold of maximum daily intake from a pasture-only diet (110-120 g DM per kg metabolic body weight), we found that the cows could achieve a DMI intake on pastures of ~15 kg DM/cow/d. This figure is close to our estimated intake. The intake rates on pasture from Paper **III** (1.8-1.7 kg DM/h, AM and PM respectively) were also in line with the meta-analysis from Pérez-Prieto and Delagarde (2013) who found most pasture intake rates to be between 1.6 to 2.4 kg



\* Data from the month of August, more representative of the quality that would be fed than July pasture.

Table 6. Nutrient composition of herbages from the four different papers. The values reported in this table are the mean between treatment and standard deviation (SD).

DM/h.
The feed quality is an important factor to understand DMI. In a PTG system, it is challenging to determine which feed the animal will favour when provided multiple sources of differing qualities. To record the effect of the treatments studied in this thesis, we consistently provided the highest quality feed possible, whether pasture or silage, in each trial. The NE of the silage was quite similar (6.0 MJ/kg DM) amongst trials (Table 3) and inferior to that of the pasture (-0.4 to -1.4 MJ/kg DM). The silages exhibited a relatively high CP content of 148 and 150 g/kg DM (Papers **I** and **IV**, respectively), surpassing the optimal level of 142 g/kg DM as suggested by Kidane et al., (2018b) for Norwegian Red cattle (Table 6). The NE of the herbage ranged from 6.4 to 7.4 MJ/kg DM, with the pasture in Paper **I** having the lowest NE content. Pasture NE contents in this thesis are at least similar, if not higher, than those found in other Scandinavian studies (Spörndly & Wredle. 2005; Kidane et al., 2018a; Kismul et al., 2018). Our values align with the early- to midmaturity grass meadow categories (006-0511 and 006-0512) in the regionally NorFor feed table (Volden, 2011), indicating high overall pasture quality. The CP content met the recommended levels for high-producing dairy cows (160-175 g/kg DM) in at least two papers (NASEM, 2021) with an unusually high content (223 g/kg DM) in Paper **IV**. The WSC content ranged from 131 to 184 g/kg DM, significantly higher than the 90 g/kg DM reported in the NorFor database for pasture grasses. In Paper **III**, we investigated the diurnal pattern of WSC content and CP:WSC ratio, which could explain the increase quality of the forage throughout the day. We were unable to record a difference between morning and afternoon sampled pastures, as seen in previous studies (Delagarde et al., 2000; Gregorini et al., 2008; Vibart et al., 2017).

The sward characteristics, such as herbage mass, availability, and sward heights, play a crucial role in pasture intake for dairy production systems (Méndez et al., 2020). Pre-grazing sward height influences herbage intake on pasture (Bargo et al., 2003). In experiments with dairy cows, pre-grazing compressed sward height was consistently >18 cm, above from those for ryegrass swards reported by Ganche et al. (2014) and Phelan et al. (2013). This overall higher pre-grazing sward height in Scandinavia is related to the lower tiller density (Virkajärvi, 2004) due to the different botanical composition in the swards. Higher pre-grazing height allows cows to select the best-quality herbage within a pasture, 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 characteristics of the swards were representative of Nordic temporary pasture grazed by dairy cows and should have not limited their intake on pasture.

Herd management such as stocking rate, pasture access, time of access, and the duration of access, are also important factors that can influence intake. The duration of pasture access was only a variable of interest in Paper **I**, where treatment groups had different access durations: 10 hours for the DAY group and 12 hours for the NIGHT group. It was considered to not affect the intake on pasture as stated in the meta-analysis by Molle et al. (2022) who examined the effects of access duration on feeding behaviour and intake. The authors concluded that herbage DMI was unrestricted when pasture access exceeds 9 h/d. The time of access was actively investigated in Papers **I** and **III** with the hypothesis that matching diurnal animal behaviour and provision of a fresh feed would stimulate intake and performance. Although the NIGHT group in Paper **I** engaged in more grazing, we did not record an increase in pasture DMI. The DAY treatment actually had a higher total DMI than the NIGHT treatment. On the other hand, when grass was more central in the feeding strategy, we found a clear effect of treatment on time spent grazing. The provision of a new evening strip did not impact the estimated DMI but impacted performances with an increase in ECM which could be related to changes in the chemical composition of the herbage as the day progressed. It is possible that the intake estimation method for the DMI was not precise enough to detect such effects in Papers **I** and **III**. A study by Soriano et al. (2001) found higher DMI in cows grazing after the evening milking compared to after the morning milking, while Sairanen et al. (2006) observed a trend for higher herbage intake in cows grazing during nighttime at high latitudes.

This collection of papers underscores the multifaceted influences on pasture DMI, including supplementation, substitution rate, feed quality, sward characteristics, and herd management. This thesis clearly shows that a crucial aspect to stimulate pasture intake was to reduce the quantities of concentrate and, more importantly, of grass silage indoor. We have provided overall good quality silage and highlighted that the grazed grass quality was consistently higher during summer. We could not prove that offering a new strip at night/evening has a positive impact on total DMI. It seems important to repeat these experiments whilst accurately measuring pasture intake to confirm the absence of effect from night/evening grazing treatment at high latitude. Relying predominantly on pasture intake (50%) in an AMS can maintain ECM production compared to indoor silage feeding with exercise pasture, yielding promising results for the ongoing debate on integrating AMS with grazing.

## 5.2 Milk performances

Milk yield is the main factor driving profitability in dairy farming and feed is the major input in livestock production. Whilst grazed grass remains one of the most cost-effective, local, and high-quality feed options for efficient dairy production (Van den Pol-van Dasselaar et al., 2018), its viability in high-output systems is increasingly questioned (Swedish Board of Agriculture, 2014). Many farmers believe that high-yielding dairy cows, especially those milked in AMS, cannot sustain high milk yields on pasture alone and instead require a full indoor feed ration to meet their nutritional needs (Becker et al., 2018; Kristensen et al., 2010).

Milk yield and milk quality recorded ranged between 22.6 and 27.7 kg MY and 23.8 to 29 kg ECM across all experiments. The best performing grazing management system was the evening new strip with 27.7 kg of milk (Paper **III**). The lowest MY (22.6 kg MY and 23.8 kg ECM) was reported with the EX treatment (Paper **IV**). The MY in Paper **I** was comparable to that in a study by Eckert et al. (2018) investigating 12 commercial barns, where cows post-peak lactation were fed a PMR in combination with grazing. The treatments of Paper **I** aimed to increase milk performances through night-time grazing, as suggested by Charlton et al. (2013) and Kismul et al. (2018). However, the latter effect was not observed due to low pasture DMI (23% of total DMI). Our results differed from Sairanen et al. (2006) who found an increase of MY of 3.9 kg/day when implementing night-time grazing in a PTG with *ad libitum* grass silage indoor. The authors attributed this effect to the higher DMI on pasture of night-time grazing cows with a higher energy content of pasture than grass silage. This suggests that to record an effect of grazing management on milk performances, the proportion of grazed grass must be significant. Overall, we were unable to record an effect of night grazing in combination to *ad libitum* PMR on milk performances. In Paper **III**, we investigated a similar treatment but this time grazed grass was the main component of the diet. In this investigation, we found that the PM treatment had numerically higher MY and daily fat yield compared to AM cows. This numerical increase, along with a significant rise in daily protein yield, led to a 10% increase in ECM for PM cows compared to AM cows. This increase was not correlated to the total estimated DMI (no difference between treatments) but may be explained by the higher quantity of better herbage quality in the evening. Similarly, Vibart et al. (2017) reported a tendency for increased milk fat, protein, and solids yield when fresh pasture was provided in the afternoon rather than in the morning. Abrahamse et al. (2009) also found a higher milk fat content in afternoon grazing rather than morning but the milk production, milk protein, and lactose content did not differ. Concentrate intake can sustain up to 1.25 kg MY/kg concentrate (Leiber et al., 2017), meaning that the pasture intake in Paper **III** has covered ~21 kg MY/cow/d. It is close to the threshold (Van Vuuren & Van den Polvan Dasselaar, 2006) of maximum production (28 kg MY/d) sustained by grazed pasture alone. The lower MY in Paper **IV** for PROD cows (-0.7 kg/cow/d) compared to the EX cows is similar to findings by Kismul et al. (2019) who found a reduction of 1.7 kg MY with a similar ECM production. The ECM yields were comparable as the difference in MY were offset by the higher percentage of milk solid (lactose) for cows in the PROD treatment.



Figure 12. Individual mean per treatment of the number of visits to the AMS, milk yield (kg/d) and total time spend outdoors (h/d) from Paper **IV**.

In AMS combined with grazing, maintaining high milk yield was a fundamental concern for dairy farmers, especially those employing grazing strategies with brief grazing periods (Kristensen et al., 2010). An approach often adopted is to increase/maintain milking frequency in AMS all year-round, as research by Stelwagen et al. (2013) and Svennersten-Sjaunja and Pettersson (2008) has associated it with improved milk yields. In Paper **IV**, the lower MY observed in the PROD treatment may be explained by the decreased milking frequency, which was 0.2 milking/d lower than in the EX treatment. Factors such as outdoor time and distance from the AMS could explain the lower milking frequency. Figure 12 illustrates that time spent outdoors did not affect milking frequency for the EX treatment, however, milking frequency decreased as time on pasture increased for the PROD treatment. The literature presents mixed findings on how milking intervals are affected by the distance between AMS and pasture and the thresholds at which changes become evident. Spörndly and Wredle (2004) observed a decline

in MY with longer walking distances to the AMS, accompanied by a 0.2 milking reduction, similar to our study. However, Kismul et al. (2018), found no significant difference in MY despite a 0.3 milking frequency variation between treatments.

The quality of the milk is essential for consumer health and purchasing decisions, and directly impacts farmer remuneration. The remuneration of the farmer in Paper **III** might increase with the offering of new strip during afternoon due to the 10% increase in ECM. The positive effect of this grazing management practice throughout the grazing season would be important to investigate. Although there is a statistical reduction in MY for PROD treatment (Paper **IV**), its practical implications are minimal. As highlighted by Steinshamn et al. (2021), feeding more forage and pasture to dairy cows led to lower MY per cow but higher profitability than farms feeding more concentrate feeds (10% reduction in PROD, Paper **IV**). The impact on profitability may therefore be minor, as farmers are compensated based on both milk volume and milk solids. Under Norway's current milk quality premium scheme, a 0.1% increase in ECM content can raise a bulk tank delivery to a higher remuneration by one level, 0.06 kr/L for protein and 0.07 kr/L for fat (TINE SA, 2024). Overrein et al. (2018) assessed factors influencing grazing profitability in Norway and Sweden and concluded that a difference in MY would need to exceed 2.8 kg milk/cow/d for profitability to favour non-pasture systems. They compared systems where cows harvested half their roughage intake from pasture to systems with an exercise paddock access only, similar to the design of Paper **IV**.

In a PTG system with *ad libitum* PMR indoor, we could not find any effect of daytime and night-time grazing on the milk production. Somewhat similar to the DMI discussion, an effect of grazing on milk performance is dependent on the proportion of grazed grass in the total diet. The milk quality can be improved by 10% with small grazing management changes such as providing a fresh new strip during the evening compared to the morning. In an AMS system combined with appropriate supplementation and high-quality pasture, the MY is reduced compared to exercise pasture but the ECM yields were maintained.

## 5.3 Dairy cows' behaviour

When kept on pasture, dairy cows' time budget consists to 90-95% of three predominant behaviours: grazing, ruminating, and resting or idling (Kilgour, 2012). About one-third of the day, 7-8 hours, is spent on grazing, and they ruminate for a similar duration; the remainder of the day is allocated to rest or idle time (Brumby,

1959). The grazing times for the animals in the present studies were 3.9 h for DAY and 4.7 h for NIGHT (Paper **I**), and 8.6 h for AM and 9.6 h for PM (Paper **III**). In Paper **I**, the animals spent between 3.0 and 3.5 h eating indoors, which accounted for approximately one-third of their available time for eating. Compared to a study by Gomez and Cook (2010) where cows were only fed indoors and spent an average of 4.3 h/d eating indoors in a commercial free-stall barn, it suggests that indoor feed access time in Paper **I** did not limit feed intake and highlights that feed ingestion was on average faster indoors (4.5 kg DM/h) than outdoors (1.1 kg DM/h). This demonstrates a cow's capacity to adapt to different feeds and orient toward the most efficient consumable feed source.

In farmed animals, natural grazing behaviours are modified and/or segmented into distinct feeding sessions due to management practices such as milking and supplemental feeding (Molle et al., 2022). All three systems explored in this thesis imposed various levels of management intensities that potentially disrupt natural grazing behaviour. Indeed, when examining allocation of activities over a 24 hour period, we found that the displayed eating/grazing patterns were influenced by the milking routine, the timing of a new daily grazing strip allocation, and the indoor PMR feeding (Paper **I**). As suggested by Molle et al. (2022), repeated feedings at the same time each day turns the feed delivery into time markers. In Papers **I** (Figure 7) and **III** (Figure 8), a surge in grazing behaviours were observed after the animals were released onto the fresh pasture. These results show that provision of a fresh grass strip can be considered a time marker. We also observed that the accumulated time grazing during the first 2, 4, and 6 hours on pasture was greater when cows were released onto fresh pasture (Paper **III**). A similar trend was reported in Paper **IV** (Figure 10) when cows in PROD treatment had a higher number of exits around 1200 h, which was the time of the day when the new strip was opened. This demonstrates the positive effect of offering feed matching with cows' motivation to graze, independent of the time of the day.

Dairy cows exhibit a diurnal grazing pattern (Rook & Huckle, 1997), meaning that there is a difference between day and night activities, with increased grazing activity during the day compared to the night (Rook & Huckle, 1997). Gregorini (2012) attributes this diurnal grazing pattern to several factors: diurnal shifts in feed quality, photoperiod, predatory instincts, and satiety hormones. Consistent with this statement, cows demonstrated a majority of their grazing/eating behaviour during daylight hours (Figures 7 and 8, Papers **I** and **III**), as also observed by Iqbal et al. (2023). We noticed a relatively brief and not particularly intense peak of grazing/eating activity around midnight. Grazing behaviour does not only follow a diurnal pattern but is also considered to show a crepuscular activity pattern that aligns intake to twilights. This has been reported in numerous studies, including Gibb et al. (1998) who noted that cows tend to graze more intensively at dawn and dusk, with the dusk period accounting for about 40% of the total daily feeding time (Taweel et al., 2004). In Paper **I**, although the significant intake of PMR indoors limited the cows' nutritional need and motivation to graze, we found that the cows in the NIGHT group still exhibited a greater grazing time  $(+0.8 \text{ h})$  and a higher grazing activity within the first two hours on pasture  $(+0.3 h)$  compared to the DAY cows. Similarly, the cows total time spent grazing was superior for the PM than AM treatment (+56 min/d), demonstrating the high grazing motivation around dusk, especially if provided with fresh pasture (Paper **III**). On average, the total time spent grazing after six hours on pasture was higher for both AM (157 *vs*. 146 min) and PM cows (228 *vs*. 124 min) during the evening feeding session compared to the morning. Interestingly, Vibart et al. (2017) observed only a positive impact on grazing when fresh pasture was offered in the morning. However, the high motivation of the cows to graze during the evening, irrespective of fresh pasture allocation time, is in line with findings by Vibart et al. (2017). The higher motivation was not only shown in total grazing time but also in grazing intensity, as a reduction in the number of grazing bouts for cows receiving fresh pasture in the afternoon with an increased duration of these bouts (+5.3 min/bout), which was also reported by Abrahamse et al. (2009) and Vibart et al. (2017). The ratio of shorter and longer wavelengths when the sun is close to the horizon has been suggested to have a stimulatory effect on appetite (Gregorini et al., 2006). The grazing peaks in both papers could also have been amplified by the milking routine (short-term fasting), as reported in other studies (Iqbal et al., 2023; Orr et al., 2001).

Cows are gregarious animals and thereby forage synchronously within their herd or flock (Rook & Huckle, 1995). This synchrony is observed when herbivores graze the same area, both in the wild or in farmed systems, often commencing a collective grazing at specific times (Molle et al., 2022). This pattern was evident in Paper **III** (Figure 8), where grazing was the main activity with low within-group variation in grazing times during the first 2 hours after returning to pasture. As presented by Molle et al. (2022), cows generally cease grazing at varying times, depending on their individual needs and/or hunger levels. This was also reflected in our study with a substantial increase in variation with increasing cumulative time (4 and 6 h), regardless of treatment.

Rumination is also an important factor to consider as it positively influences milk production (Beauchemin, 2018) and its main drivers are intake, chemical and physical characteristics of the diet. The total daily rumination time of cows in the Papers **I** and **III** were within the range (387-530 min) reported by Pérez-Prieto and Delagarde (2012). In Paper **III**, we observed the occurrence of multiple grazing sessions between sunrise and sunset, interspersed with intervals of rumination, as stated by Gibb et al. (1998). Moreover, most of the rumination occurred at night, as also stated by Rook and Huckle (1997). Interestingly, cows in the PM group showed a shorter total daily rumination time than cows in the AM group (Paper **III**). This difference could be due to greater digestibility (Linnane et al., 2001) and palatability (Provenza et al., 1998) of the pasture, or/and to more selective grazing behaviour by the PM group leading to a similar effect. Notably, the aNDF content of the herbage might have explained the rumination reduction as it was numerically lower in the PM pasture (338 g/kg DM) than in the AM pasture (369 g/kg DM). Gregorini et al. (2009) reported a decrease in toughness of meadow fescue, and an increase of particle size reduction, from early morning to evening because of a relative decrease in fibre concentration in the herbage and an increase in DM and WSC content during the day. Although there was a numerical difference in the WSC:CP ratio between the AM and PM pastures, we did not find a statistical significance in the difference and therefore can only speculate around this effect. Differences in grazing and rumination behaviours have been previously associated with the stage of lactation and parity, due to the different energy requirements in the various physiological states (Iqbal et al., 2023). However, stage of lactation and parity did not influence grazing or rumination duration in Paper **III**. However, we found an effect of stage of lactation on production parameters and parity is known to impact the time budget of dairy cows (Grant & Albright, 2001). The cows on the organic farm have been specifically bred to cope with an intense full-time grazing system and were acclimatised to grazing for more than a month prior to our sampling, which could possibly explain the lack of parity effect.

Indoor and outdoor times were recorded in the Paper **IV**. In this trial, the pasture serves two purposes: feeding or exercising. The cows in the PROD treatment spent significantly more time outdoors (+6.8 h) than EX cows, a direct consequence of their necessity to graze for their nutritional requirement. As displayed in Figure 10, the highest outdoor activity (4 to 6 h spent outdoor per exit) occurred between midnight and 0600 h, suggesting a preference for cooler night-time conditions on pasture. It is likely that both groups used the pasture as a resting area during the night, and it has indeed been shown that cows are motivated to visit the pasture for resting at night (Smid et al., 2018). The temperature humidity index (THI) might explain the cows' preference of spending the night outdoors throughout the

experiment. The recorded THI never exceeded the heat stress threshold for lactating dairy cows (Kibler, 1964), but THI were consistently lower on pasture, and it is known that cows may alter the behaviour to avoid heat even in temperatures below the threshold (Lovarelli et al., 2024). Regarding the current and future increasingly extreme weather, evening/night grazing or outdoor exercise may gain prominence for its potential benefits of providing the cows access to a valuable resource without negatively affecting their wellbeing. By offering pasture access at night, we can enhance grazing or resting conditions for the cows with cooler temperatures and diminished solar radiation.

Whilst the provision of fresh pasture serves as a strong motivator for grazing behaviour, our findings indicate that cows predominantly engage in grazing during the afternoon, irrespective of whether they receive a morning or afternoon fresh strip. Aligning provision of fresh pasture access with this natural diurnal pattern has the potential to significantly increase ECM production. For farmers already utilising rotational grazing management (e.g. strip grazing), implementing this grazing modification is straightforward, low cost, and does not increase workload.

### 5.4 Enteric methane emissions

Reducing eCH<sup>4</sup> emissions presents a serious challenge in grazing systems (Vargas et al., 2022). Mitigation strategies need to be efficient and persistent, safe for both animals and consumers, and economically viable and practical in order to be adopted by farmers. Enteric CH4 emissions mitigation must be achieved without decreasing or even improving animal's performance (quantity and quality of products). This implies an evaluation of strategies with the primary focus CH4I per kilogram of product (MY or ECM) for farmers. Investigating a nutritional strategy to mitigate eCH4 emissionsis highly relevant as it is more rapidly applicable compared to other strategies such as animal genetic selection or new feed additives. Numerous nutritional factors can influence eCH4, but quantity and digestibility of intake are the key factors (Knapp et al., 2014). In Scandinavia, most of the DMI is composed of conserved forages (grass silage) throughout the year and its quality is usually lower than grazed grasses in summer. Koning et al. (2022) found that the Tier 3 model (Bannink et al.,  $2011$ ) generally aligned with actual CH<sub>4</sub> measurements, but discrepancies occurred when applied to grazing cows. The model fails to accurately reproduce eCH<sub>4</sub> emissions for cows on pasture, even when accounting for DMI, ration composition, and observed *in situ* degradation characteristics of feed. If intake of fresh forage yields lower  $eCH_4$  emissions in grazing, this may be a strong argument to promote grazing (and not only pasture access) in feeding systems management. To investigate the potential difference in eCH<sup>4</sup> between herbage intake from pasture and grass silage intake within a day, we investigated the idea of installing two GEM units, one indoor and one outdoor (Papers **I** and **IV**).

#### 5.4.1 Enteric methane emissions of different feeding systems

The absolute  $eCH_4$  emissions values reported in this thesis are in line with those documented in the literature for dairy cows of equivalent production level. The eCH<sup>4</sup> emissions from the dairy cows averaged between 326 to 387 g CH<sub>4</sub>/d, the CH4Y between 16.5 to 24.5 g CH<sub>4</sub>/kg DM and the CH<sub>4</sub>I between 11.8 to 18.5 g CH<sub>4</sub>/kg ECM (Table 3). In their meta-analysis from European studies, Appuhamy et al.  $(2016)$  reported that daily eCH<sub>4</sub> emissions from dairy cows ranged from 251 to 498 g CH<sub>4</sub>/d (376 g CH<sub>4</sub>/d on average), from 15.5 to 26.3 g CH<sub>4</sub>/kg DMI (21.3 g CH<sub>4</sub>/kg DMI on average), and from 10.5 to 32.3 g CH<sub>4</sub>/kg milk (16 g CH<sub>4</sub>/kg milk on average). The eCH4 emissions recorded indoors were consistent with findings by Ramin et al. (2021) (384–504 g CH4/d) in Paper **I** and slightly lower in Paper **IV**, whilst the outdoor  $eCH_4$  emissions were consistent for both papers with values found by Waghorn et al. (2016) for cows on pasture (240-360 g CH4/d). In Paper **III**, the CH<sup>4</sup> emissions were not measured but estimated according to the equation of Niu et al. (2018) based on intake and NDF content. A recent meta-analysis by Brito et al.  $(2022)$  compared eCH<sub>4</sub> emissions measured and predicted, also based on Niu et al. (2018), in dairy cows under confinement and grazing systems. The authors showed that eCH<sub>4</sub> emissions were significantly lower in grazing systems without a difference in CH4Y and CH4I due to a reduction of milk performance. The authors also observed a more pronounced reduction in  $eCH<sub>4</sub>$  emissions with grazing systems compared with confinement systems, due to a 19%  $g$  CH<sub>4</sub>/d overestimation of the predicted eCH<sup>4</sup> values compared with measured values. Given this potential overestimation, the CH<sub>4</sub> estimates from Paper III may be more comparable to those reported in Norway by Kidane et al. (2018a), ranging from 263 to 327 g CH $_4$ /d with similar milk performance (270 – 273 g CH4/d if reduced of 19% for Paper **III**).

Many authors have reported lower  $eCH_4$  emissions in grazing dairy cows than those fed TMR (Cameron et al., 2018; Civiero et al., 2021; Mufungwe et al., 2014; O'Neill et al., 2011). These authors also observed a reduction of eCH4I which was sometimes accompanied by a reduction of MY due to lower intake (intake effect) but the eCH<sup>4</sup> emissions were reduced to a greater extent (diet effect), resulting in significantly lower CH4I in grazing compared to TMR.

Given that PTG systems blend feeding characteristics of both grazing and TMR, it raises the question of how their  $eCH_4$  emissions and intensities compare. Dairy cows in PTG systems studied in this thesis (Papers **I** and **IV**, PROD) emitted less eCH<sup>4</sup> and had lower CH4I compared to recordings of cows fed TMR (Civiero et al., 2021; Dall-Orsoletta et al., 2016) and silage only (Paper **IV**, EX). These metrics are also higher than the estimated  $eCH_4$  and  $CH_4I$  than in the full grazing system from Paper **III**. A study comparing PTG and TMR, found that PTG exhibited significantly lower eCH<sup>4</sup> emissions and CH4I compared to those fed TMR. O'Neill et al. (2012) found an increase of  $eCH_4$  when supplementing a PMR (silage + concentrate) compared to full grazing but a decrease in CH4I. This reduction was explained by higher MY; however, the uneven comparison arose as the grazing treatment did not receive any concentrate. Dall-Orsoletta et al. (2016) reported significantly lower eCH4, CH4Y, and CH4I when cows in a PTG system had grass included in their diet (38 to 45% grass intake) compared to TMR only.

The effect of the treatments (and so pasture or silage inclusion in the diet) on recorded CH<sub>4</sub> emissions highlighted that the lowest eCH<sub>4</sub> emissions and CH<sub>4</sub>I recorded was the treatment with the highest pasture intake (PROD). In contrast, the highest  $eCH_4$  and  $CH_4I$  observed was the treatment with the highest grass silage intake (EX) (Paper **IV**). In their PTG trial, investigating unrestricted grazing (full grazing) vs restricted grazing (PTG), Koning et al. (2022) found that the inclusion of silage, on average over two years, increased the  $CH_4Y$  by 3.5 g  $CH_4/kg$  DMI. The regression from this thesis showed a similar trend but with a smaller amplitude (1 g  $CH<sub>4</sub>/kg$  DMI of silage). The reduction in intensity between the PROD and EX treatment was important, with 27% less CH<sup>4</sup> per kg of ECM. The multi-years trial from Koning et al.  $(2024)$  confirms the trend with the eCH<sub>4</sub> emissions lowering alongside the increase in proportion of pasture in the diet. The authors recorded eCH<sup>4</sup> emissions and CH4I from feeding systems with various levels of pasture intake and they ranked, from lowest to highest, as such: unrestricted grazing < restricted grazing  $(PTG)$  < zero grazing (cut and carry) < grass silage feeding, at the same concentrate levels. When using both indoor and outdoor GEM units, a confounding effect between the method (GEM units) and the treatment (diet effect) can arise. However, in Paper IV, the diet effect can be isolated, showing reduced eCH<sub>4</sub> emissions, with fresh grass intake, and these were lower for PROD cows  $(357 \text{ g CH}_4/d)$  compared to EX cows (387 g CH4/d) when measured on the same indoor GEM unit.

A dietary shift from grass silage to grazed grass in the diet seems to indicate a reduction of eCH<sub>4</sub> emissions, highlighting the potential of fresh grass inclusion in the diet to lower emissions. We have been able to directly record this shift of

emissions when an animal's changed diet with their environment (indoor *vs* outdoor). In Papers **I** and **IV** (PROD), the cows were recorded emitting less eCH<sup>4</sup> emissions on the outdoor GEM unit than on the indoor GEM unit. Overall, we observed a decrease in eCH4 emissions ranging between 28% and 20% (Papers **I** and **IV**, respectively) when the cows were eating fresh grass outdoors compared to when they were recorded eating grass silage indoors. A reduction of the same amplitude (- 28%), was reported by Koning et al. (2022) when cows were consuming grass in their PTG trial, while Denninger et al. (2019) reported an increase (+30%) in emissions when cows were moved from a summer pasture to a winter barn. When plotting eCH $_4$  against the proportion of herbage in the diet from this thesis, a 10% increase in herbage proportion results in an  $8\%$  reduction in eCH<sub>4</sub> (R<sup>2</sup>=0.79). Similarly, an increase in herbage intake from pasture of 4 kg DM in total DMI decreased CH $_4$ I by 1 g CH<sub>4</sub> per kg ECM ( $R^2=0.60$ ). Despite higher total DMI and MY in the study by Koning et al. (2022) compared to Paper **IV** for a similar treatment, CH<sub>4</sub>I values were comparable.

We found evidence that the fermentation in the rumen of grazed grass can lower by 20-28% the eCH<sup>4</sup> emissions compared to grass silage. The amplitude of reduction of CH4I was also lower (-28%) when herbage was fermented in PTG compared to grass silage. To reduce the eCH<sup>4</sup> emission in a PTG, increasing pasture intake at the extent of grass silage seems to be a valid feeding mitigation strategy. Adjusting emission factors for forages to reflect the lower values for fresh grass compared to grass silage would better capture the environmental benefits of grazed grass.

#### 5.4.2 Behind enteric methane emission differences

The difference in forage quality can largely explain the lower  $eCH_4$  emissions from cows grazing fresh grass compared with cows eating conserved grass. The main factor of quality influencing the methanogenesis potential of a feed is the OMD (Sauvant & Noziere, 2016). High-quality forages, such as early development plants, have high contents in easily fermentable carbohydrates and low NDF content, thus enhancing digestibility and accelerated particle passage rate in the rumen and, consequently, the time of exposure to fermentative microbes. This overall better quality of grazed grass compared to the grass silage was evident in Papers **I** and **IV**. This effect can also be found between grasses of different vegetation stage as see in the *in vitro* experiment (Paper **II**), where the OMD of the pasture was in August (76%) rather than in July (68%). This has led to a lower methanogenic potential of feed incubated. This trend was confirmed by the higher content of NDF positively correlated to CH<sub>4</sub> production ( $\mathbb{R}^2 = 0.76$ ). We also found that the total VFAs were not influenced, but a tendency of lower acetate and propionate molar proportions was found, resulting in a lower acetate to propionate ratio in the August herbages. This lower ratio confirms that  $H_2$  had been used for the propionogenesis at the expense of methanogenesis.

Both Koning et al. (2022) and Cameron et al. (2018) found that eCH<sub>4</sub> emissions and CH<sub>4</sub>I were lower in grazing treatments compared to zero-grazing systems (cutand-carry). This discrepancy may arise from feed alterations (during cutting height and time before feeding) and from the higher quality of herbage consumed directly by grazing animals compared to harvested forage. Moate et al. (1999) showed that, regardless of herbage allowance, cows selected a diet approximately 10% higher in *in vitro* DM digestibility, 30% higher in CP, and lower NDF content than the herbage on offer. The overall better digestibility of the grasses compared to the silage shifts fermentation towards propionate and improves milk production.

The study from Koning et al. (2024), which spanned over several years, suggests that the CH4Y from fresh grass may depend on the proportion of silage grass in the ration, potentially due to the slower passage rate of grass silage in the rumen. This contrasts with the typical effect observed with concentrates, where their high digestibility increases passage rate and decreases NDF digestibility, suggesting that the combination of easily digestible fresh grass and slower-digesting grass silage may result in higher CH<sub>4</sub> production. In addition to the classic characteristic of the chemical composition, Koning et al. (2022) hypothesised that wax n-alkanes could limit the fermentation of sugar in the rumen by providing more resistance, causing some of the sugars to only be released in the intestine, thereby limiting the sugar potential to be fermented in the rumen.

Although the specific causes behind the lower measured  $eCH_4$  emissions from fresh grass were not the primary focus of this thesis, we consistently recorded lower eCH<sup>4</sup> emissions on pasture in the two papers investigating it and provided interesting hypothesis for future investigation. The consistently higher-quality of the herbage compared to the grass silage in summer, is one of the key factors driving these reduced eCH<sup>4</sup> emissions. Finding the optimal balance of pasture and grass silage is crucial to achieve a ration that minimises CH $_4$ I and CH $_4$ Y while maintaining performance. The results of this thesis emphasise the potential mitigation benefits by directly utilising pasture, instead of transforming it into grass silage as a summer feed for the next year in Scandinavia.

## 5.5 Critical analysis of experimental designs and methods

#### *Experimental design*

The different experimental sites, four different farms, have their advantages and disadvantages. On one hand, it reflects diverse grazing management systems at various latitudes, providing a broader understanding. On the other hand, the constant resetting required to understand and operate each new site was challenging. Whilst simpler experimental designs can benefit from multiple locations, more complex experiments, such as with  $eCH_4$  emissions measurements combined with grazing experiments, may be more effectively conducted in a single experimental barn. The quality of the information obtained from Paper **III** is, to a large extent, due to the farmer's knowledge and grazing experience.

The design of the experiments could also have been improved. A change over design should have been preferred to strengthen the reliability of the conclusions from the results of the treatments in Paper **I**. It would have been very complicated in Paper **III,** as experimenting at a commercial farm is challenging. The implementation of a complex experimental design could disturb the farmers production and economic performance. An increase in the length of the recording period could have been a path to improvement. After learning from previous experiments, we chose to implement a changeover design and increase the recording period by one week in Paper **IV**. Undertaking comprehensive research on grazing and eCH<sup>4</sup> emissions over a prolonged period poses considerable challenges, requiring a significant investment of resources, including knowledge, experience, and infrastructure. It is possible that we underestimated the necessary resources at the beginning of the study, which may have impacted the quality of the study design and results. Nevertheless, our competencies in this area have substantially improved over the course of the research.

#### *Recording pasture intake*

Recording intake is relatively straightforward indoors, where a variety of feed bunk technologies, such as the RIC system (Roughage Intake Control<sup>TM</sup>, RIC, Insentec B. V., Marknesse, The Netherlands; Paper **I**) and BioControl system (CRFI Feed, BioControl, Rakkestad, Norway; Paper **IV**), which automatically track feed consumption, are available. However, recording intake for grazing animals is more challenging. There are several direct and indirect measurements techniques. The disappearance technique is a commonly used and simple method to directly measure intake under grazing conditions (Mayes & Dove, 2000). In the sheep study (Paper

**II)**, this technique produced realistic DMI figures, comparable to results from (Åby et al., 2023), and Lind et al. (personal communication). This method is more accurate in homogenous pastures at a relatively shorter sward height. However, due to high pre-grazing sward heights and a methodological oversight (failing to establish a postgrazing herbage mass regression), DMI may have been overestimated in Paper **II**. This overestimation is likely due to higher stem bulk density and lower leaf bulk density in the lower part of the forage, which affected DM density. This effect may have been more pronounced in August when forage height after grazing was at its highest. To ensure the validity of our results, we estimated intake with the INRAE formula (INRA, 2007) and obtained similar DMI values for July and confirmation of overestimation in August (-0.7 kg DM) compared to the disappearance technique.

Back calculation from energy requirement is an alternative method to estimate intake, and it is also less labour demanding and more economical. A common approach in grazing studies is to back-calculate intake by comparing known intake with calculated requirements. This technique was applied across all four papers in this thesis, as our expertise and resources to measure grazing intake were limited. However, this method introduced several uncertainties, beginning with the choice of equation for calculating animal requirements: INRAE (INRA, 2007), NASEM (NASEM, 2021), or NorFor (Volden, 2011) and the nutritive quality of the feeds among others. In Papers **I** and **III**, we have estimated the intake using several equations and selected the best fitting one by looking at the rumen fill and energy balance values. The updated equation from NASEM by De Souza et al. (2019) was the most appropriate model for both trials. The results showed that using the same methodology highlights the versatility of this technique across two different management systems, yielding similar production results. However, they remain estimates, and caution should be exercised when interpreting them. In Paper **IV**, the DMI concordance between the two treatments further substantiates the biological validity of the method used for the PROD treatment since the DMI from EX treatment was recorded and estimates were similar (3% of difference).

#### *Measuring enteric methane emissions on pasture*

Quantifying eCH<sup>4</sup> emissions, particularly in grazing systems, presents significant challenges. A variety of global technologies, each with its unique application, cost, and accuracy, are utilised to measure individual  $eCH<sub>4</sub>$  emissions. However, all direct methods rely on evaluating the flux of CH<sup>4</sup> erupted. Johnson et al. (1994) pioneered the first technique, the  $SF_6$  tracer technique, which unlocked the possibility to document grazing eCH<sup>4</sup> emissions in ruminants. The development of a functional system using the  $SF<sub>6</sub>$  tracer technique was performed in Paper **II**. The system of gas

collection was designed during the spring of 2020. Developing a functional  $SF<sub>6</sub>$ system requires significant time and preliminary trials to ensure its effectiveness. In my opinion, the project was not feasible under our initial conditions without expert guidance during its development. We encountered numerous technical challenges, primarily due to the lack of gas chromatography (GC) equipment in the laboratory to analyse gas concentrations  $(SF_6, CH_4)$  in air samples collected from the animals. Key issues included: canister pollution with the  $SF_6$  resulting from  $SF_6$  bolus incubation and canister cleaning in the same room, and the inability to immediately analyse the gas concentration in canister post-collection (incapacity to troubleshoot in time). Additionally, the vial nitrogen dilution prior to transportation to the laboratory for GC analyses should have been performed but was overlooked, resulting in many empty glass vials.

The *in vitro* technique was then selected to quantify the methanogenesis potential of the herbage collected during the two months of experiment. The *in vitro* technique and CH<sup>4</sup> prediction has faced criticism as its results are not always replicable *in vivo* (less clear mitigation *in vivo* than *in vitro*) and tend to overestimate the CH<sup>4</sup> emissions (Yáñez-Ruiz et al., 2016). However, the main criticism about Paper **II** in my opinion is that we tried to "save" the experiment without taking the time to redefine the objectives. An alternative objective could have been to remove the *in vivo* data and focus on the methanogenesis potential of feed representing the yearround feed: silage during the winter month, infield pasture during the early and late grazing period and herbages grazed by the sheep in the utmark. This approach could have yielded more insightful results whilst better leveraging the strengths of the *in vitro* method (screening) to assess the year-round methanogenic potential of the sheep sector, avoiding the high costs and challenges of an *in vivo* trial.

#### *The GreenFeed method*

The GreenFeed® system (C-Lock; GEM) has gained considerable popularity in recent years due to its numerous advantages. These include suitability for installation in both experimental and commercial barns, indoor and outdoor measuring capacity, high measuring animal capacity per unit (n= 20-30 cows/GEM unit) in their productive environments, its mobility, and minimal animal discomfort. The GEM unit is a spot-sampling technique requiring a minimum of 20-30 voluntary visits per cow and treatment to gain a representative  $eCH<sub>4</sub>$  value for significant effect detection, equating to 7-14 days of recordings (Manafiazar et al., 2016). A minimum number of voluntary visits is therefore vital to obtain reliable data. We have rarely reached this threshold at the individual level, but it was reached at the treatment level. We have noticed that certain animals would rarely or even never visit the GEM

unit, as highlighted by Waghorn et al. (2016) and Hammond et al. (2016). This phenomenon is more common with grazing animals compared to animals kept indoors. This difficulty was more pronounced in the Paper **IV** compared to Paper **I**.

A common technique to increase the visitation is to intensively train the animals prior to the experiment or to select already trained indoor animals for the experiment. The training is time consuming and is unfortunately not always fruitful. There is currently no documentation on the best training practices and strategies to enhance visitation. Despite over three weeks of intensive training in Paper **I,** with numerous personnel involved, six cows never learned to visit the GEM unit frequently enough. The design of the barn is also important because the training was less difficult when cows were locked on pasture for a certain time and easily attracted to the GEM unit (Paper **I**). Contrary, in the AMS barn, the training period was shorter (2 weeks), and cows were more difficult to bait since they were free moving between indoors and outdoors. I would therefore advise to start the training time much earlier and for a significantly longer period in order to achieve reliable visitation to the outdoor unit in an AMS system set up.

To the best of my knowledge, our work is the first published study using two GEM units online in different environments (indoor and pasture). Only the scientific reports by Klootwijk and Koning (Klootwijk et al., 2021; Koning et al., 2024; Koning et al., 2022) have used the same methodology in some of their trials. Overall, this method has shown promising results and could potentially improve the understanding and management of  $eCH<sub>4</sub>$  emissions from cows in PTG or grazing with silage supplementation. Based on the experience gathered during Papers **I** and **IV** with the two GEM units, we can propose several technical recommendations: 1) the proximity of the GEM unit to the animals on pasture increased the number of visits (Paper **I** compared to Paper **IV**). In the first trial, the GEM unit was moved daily to the next strip alongside the water trough and mineral block, creating a relatively close area of interest for the animals. This movement was repeated only every 2-3 days in the Paper **IV** as a strip forward grazing management was employed. Therefore, it can be hypothesised that the relative distance between the animal and the GEM unit on pasture may impact the visit frequency of the animals; 2) The time of access seemed to be an important parameter that can influence the visit frequency to the outdoor unit (0.9 visit/d for DAY and 1.6 visit/d for NIGHT, Paper **I**); 3) Operating the GEM units as two different systems is easier as you can individually modify certain setting on the units. This enhances the capacity to increase the maximum visits on the unit of choice.

# 6. Conclusions

The overall aim of this thesis was to investigate different dairy cow grazing management systems in Nordic conditions, characterised by unique high latitudes farming. The evaluation centred on dairy cows' performances (intake, milk quantity and quality), behavioural responses, and  $eCH<sub>4</sub>$  emissions from consumption of herbage on pasture and grass silage indoor.

Forages constituted the core feed of dairy cows' diet in summer with grass silage and fresh grass representing more than 58% of the total DMI. The various levels of herbage intake in the diet studied, from 20% to 76% of total DMI, demonstrated the plasticity of this feeding strategy to different barn environments. The feasibility of designing a system that predominantly relies on grazing (>50% total DMI) during summer is certainly achievable, even in AMS farms.

When grazing intake is minimal, grazing management treatments (e.g. night-time grazing) had no effect on intake and milk performance. However, when grazing is more prevalent in the feeding system, milk performance increased by 10% in terms of ECM. It was also possible to maintain ECM production in AMS farms with half of the intake coming from production pasture compared to indoor feeding (exercise pasture).

Dairy cows grazed for a longer duration in the evening regardless of treatment, representing an opportunity to increase their feeding duration. Making minor changes to grazing management, such as aligning the supply of fresh feed with the animals' behavioural preferences, yields promising results if grass consumption remains the main source of feed in the animal's diet.

The differences in the chemical composition between grass silage and grazed grass have been found to affect  $eCH_4$  emissions. With this dietary shift from grass silage to grazed grass, the ECM was maintained and CH4I was reduced demonstrating a direct effect of high forage quality on the  $eCH<sub>4</sub>$  emissions from dairy cows. The eCH<sup>4</sup> emissions from dairy cows eating herbage on pasture were 20 to 28% lower compared to those eating grass silage indoor.

The deployment of two GEM units both indoors and outdoors was relevant for investigating eCH<sup>4</sup> emissions in PTG systems whilst capturing the potential differences in emissions from feeds of various quality consumed. Intensive training of the animals and extending the recording period could address the main challenge with this method, which was ensuring that the animals consistently visited the outdoor unit. This method was more challenging to set up in an AMS system due to the increased difficulty in training the cows and their capacity to move freely between barn and pasture.

Overall, this work underscores the positive potential of providing pasture for dairy cows, which not only enhanced animal welfare but also reduced eCH<sub>4</sub> emissions whilst maintaining milk performances. This approach could help to reduce the climate footprint of the dairy sector in Scandinavia.

# 7. Future perspectives

Looking towards the future of dairy systems in Scandinavia, there are several exciting prospects and avenues for exploration. Regarding grazing dairy cows in Norway and Sweden, it seems important to document and report more information about grazing management and gain more reliable grazing intake data during summer. Without more precise data, it will be quite difficult to assess the situation. It is also important that more research is conducted to understand the fermentation processes of fresh grass and its interaction with grass silage in the diet of ruminants. Regarding eCH<sup>4</sup> emissions, it appears important to investigate the effect of implementing several strategies, as advised by Arndt et al., (2022): is there a cumulative effect or not, and the interactions, applicability, and compatibility between different strategies.

The substantial technological advancements in recent years have significantly transformed the dairy sector, with innovations like AMS being prime examples in Norway. A connected dairy barn is producing a gigantic amount of data: intake, behaviour, body weight, position, milk yield, quality, weather, data with drones and satellites. One of the main challenges facing precision livestock farming is now merging these technologies' data to obtain more information.

For instance, virtual fencing could be deployed with productive dairy cows to create more efficient grazing systems by aligning with their behavioural preferences. A new strip could be automatically offered once a day when cows display recurrent high grazing behaviour, to optimise intake on pasture. Further investigation into positive cues in virtual fencing could also be beneficial. If connected to the AMS, the collar could potentially deliver a "positive" cue (different sound effects) to encourage the cows to return for milking when reaching a time threshold. This integration of technology could create a more efficient, responsive, and animal-friendly farming system.

- In general, the development of tools to easily monitor intake on pasture seems crucial. The utilisation of technology recording behaviours (e.g. NEDAP) to estimate the intake of the animal on pasture seems a promising avenue. It would allow farmers to be more confident and efficient in their indoor supplementation.
- In terms of  $eCH_4$  emissions, there is a vast potential for using proxies to offer more practical and accessible methods for monitoring and mitigating emissions at the farmer level. During this work, a collaboration has been initiated with the Walloon Agricultural Research Centre (CRA-W) to estimate eCH<sup>4</sup> from the milk mid-infrared (MIR) spectral information. The provision of milk MIR spectra data from Scandinavian cows (Paper **IV**) were used to estimate CH<sub>4</sub> using their existing model constructed from  $SF<sub>6</sub>$ and respiration chambers data. The results were not good (poor correlation), especially the outdoor deviation. This confirms that: 1)  $eCH<sub>4</sub>$  prediction models based on milk MIR spectra are technically dependent, hence the ongoing construction by the CRA-W of a prediction model based on GreenFeed data; 2) sharing data with CRAW will increase the model's range of validity by integrating data not previously represented in their model (Scandinavian breeds, pasture GEM data, AMS). I think that the development of these proxies is crucial for providing farmers with reliable data. Given the urgency of climate change, it is essential for farmers to receive recognition and incentives for adopting environmentally sustainable production practices.
- The development of Low Tech, defined as simple to use and manufacture, could be of growing interest in the future. It could accelerate certain transitions, as they are generally cheaper, not overwhelming in data quantity and easier to adopt. Complementarity between Low and High Tech is an interesting prospect in a world with limited resources. Not all aspect of the production necessitate huge amount of data.

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Photo: Therese Jægtvik

# Popular science summary

The dairy industry in Norway and Sweden has become more intensive, leading to less reliance on grazing as the main feed for cows. This trend can be seen across Europe, whilst the livestock sector faces growing pressure to reduce its climate impact. A major concern is the emission of enteric methane  $(eCH<sub>4</sub>)$ , a greenhouse gas produced by cows during feed digestion in the rumen.

This work explored how optimisation of grazing during summer could affect the milk production of dairy cows and eCH4. This is implied to replace indoor grass silage with grazed grass in dairy cow's diet. To investigate eCH4 emissions between cows grazing and cows fed silage indoors, we used a unique setup, two machines that measure eCH<sup>4</sup> emissions directly from cows, which were installed outdoors and indoors. The results showed that forages in the diet, including grass silage and fresh grass, made up over 58% of the cows' total feed during summer. We found that diets with 20% to 76% grazed grass could be an efficient strategy on performances of different dairy barn systems. Even farms with automatic milking systems can rely on grazing for over 50% of the cows' summer diet whilst simultaneously maintaining the production performances compared to only indoor feeding. When grazing was more prevalent in the diet, night-time grazing improved milk quality by 10%. Cows naturally grazed longer in the evening and offering new fresh feed at this time provided promising results. At the same time, replacing grass silage with fresh grazed grass reduced  $eCH_4$  emissions by 20% to 28% whilst maintaining milk performances.

Optimising summer grazing for dairy cows in Scandinavia can not only improves the animal's diet and could also reduce  $eCH_4$  emissions and, simultaneously, help improve the sustainability of dairy farming.



Photo: Jorid Sandvikk

# Populärvetenskaplig sammanfattning

Mjölkproduktionen i Norge och Sverige intensifierats under de senaste årtiondena, vilket har lett till att bete som en del i foderstaten till kor har minskat. Genom att erbjuda en foderstat till korna som baseras på inomhusutfodring har man lättare kontroll över att de får i sig rätt mängd foder för att kunna producera mjölk på önskad nivå. Trenden med minskad andel bete i foderstaten kan ses i hela Europa, samtidigt som kraven på att minska klimatavtrycket från boskapssektorn ökar. En av de mest potenta växthusgaserna är metan (CH4) som produceras av mikroberna i kons första mage vommen under fodersmältningen, och som korna rapar upp när de idisslar.

Detta arbete undersökte om bete i foderstaten till mjölkkor kan användas för att reducera metanutsläppen från korna. Detta innebär att ersätta inomhusensilage med betat gräs i mjölkkornas diet. För att undersöka metanutsläpp hos kor som betar och hos kor som utfodras med ensilage inomhus använde vi en unik uppställning med två maskiner som mäter metanutsläpp direkt från korna, installerade både utomhus och inomhus. Maskinen består av ett bås med en krubba där korna ges lite kraftfoder. Korna besöker frivilligt båset, och när kon står i båset samlas utandningsluften in, och i den mäts mängden metan. Resultaten visade att korna producerade samma mängd mjölk när foderstaten innehöll 50% bete jämfört med när de fick all sin mat inomhus. Korna betade i längre perioder på kvällen än på morgonen och förmiddagen, vilket är i linje med deras naturliga beteende. Genom att erbjuda nytt bete på kvällen när korna är som mest motiverade att beta, minskade metanutsläppen med över 20%, jämfört med de kor som fick all sitt foder inomhus, samtidigt som avkastningen var densamma i båda grupperna.

Att optimera betesintaget för mjölkkor i Norge och Sverige har stor potential. Genom att inkludera bete i foderstaten främjas kornas naturliga beteende samtidigt som metanutsläpp minskas utan negativ påverkan på avkastningen. Tillsammans leder detta till en förbättrad hållbarhet inom mjölkproduktionen.



The island of serenity (Steinkjer) Original illustration from Olivier Martin

# Acknowledgements

This project was a collaborative work between the Norwegian Institute of Bioeconomy (NIBIO) and the Department of Animal Nutrition and Welfare at the Swedish University of Agricultural Sciences (SLU). I would like to express my gratitude to the Norwegian Institute of Bioeconomy Research, the Swedish University of Agricultural Sciences, Trøndelag Fylkeskommune, and the Steinkjer municipality for their generous funding that made this PhD possible.

My appreciation goes to my main supervisor **Mårten** Hetta (SLU) for his support throughout this PhD adventure. I am grateful of the support and guidance of my cosupervisors, **Mohammad** Ramin, **Mats** Höglind, **Vibeke** Lind, and **Haldis** Kismul. It was not always an easy journey due to scientific isolation, but I am thankful for your insights and guidance.

I would like to thank the people that helped me on the kappa by providing feedback and helping me find a red thread, by making illustrations and encouraging me throughout this last step of the PhD: **Mårten** Hetta, **Mats** Höglind, **Rebecca** Danielsson, **Emma** Ternman, **Olivier** Martin, my **mother** and **sister**, and **Annabel**.

My great appreciation goes to **Agnes** van den Pol-van Dasselaar for participating at my half time seminar and providing valuable feedback, and for accepting to be the main opponent at my PhD defence. I would like to also thank the jury members, **Alberto** Stanislao Atzori, **Deirdre** Hennessy and **Jon** Moen for accepting to review my work and for their presence at my defence.

I am grateful for the time dedicated by my colleagues for day-to-day help in setting up and monitoring experimental work on the various farms, and laboratories:

• At **Tjotta research station**: **Grete** Jørgensen, **Ievina** and **Robert** Sturite, **Shelemia** Nyamuryekung'e, **Thomas** Karacs and everyone else. Thank you for welcoming me on my arrival in Norway and for your support through the trials.

- At **SLU Umeå: Reija** Danielsson, **James** Mushi, **Stig** Brodin, **Jenni** Burman, and all the French students that helped us, especially **Amelie** Laval. Thank you for your support in running the trial in the barn. My gratitude also goes to **Ann** Sophie, **Jeanette** Nilsson, **Mohammad** Ramin, and others for helping to run the *in vitro* trial.
- At **SLU Uppsala Astrid** Gumucio and **Maria** Åkerlind: thank you for analysing our samples and helping us with dry matter estimates:
- At **Koinge:** a huge thanks to **Martin** and his family for sharing their enthusiasm for grazing and ice cream. It was an amazing collaboration and a great opportunity to make a trial in this barn.
- At **Maere landbruksskole**: **Tove** Jystad, **Rolf** Kristian Wensbakk, **Arnulv** Vestrum, **Ingrid** Barstad Saltvik, the other barn personnel, and the students, thank you for welcoming us in your barn. and helping during the trial.
- At **Nord University**: Thank you to everyone who helped me in providing answers to my numerous questions and always warmly welcomed me.

I would like to thank all my co-authors for their contribution to the valorisation of results, scientific discussions, and editing of publications. I am very grateful to the wonderful people that helped us in the design of the Umeå trial and welcomed me in the Ekofrost project to work on the Koinge paper. A particualarly huge and special thanks to **Rebecca** Danielsson**, Emma** Ternman, and **Horacio** Gonda! Thank you for your guidance, friendship, and help that extended beyond the paper. I would like to thank the collaborators with whom I worked with during this thesis: **Amelie** Vanlierde (CRA-W) and **Clement** Grelet (CRA-W) for the milk spectra collaboration; **Yvanne** Rochette (INRAe) and **Peter** Dörsch (NMBU) for the support on analysing and troubleshooting of the SF6 results; **Karoline** Bakke, **Sverre** Lang-Ree and **Eli** Hveem Krogsti (GENO) for allowing us to use their equipment.

A special thanks to all my colleagues at Steinkjer NIBIO for welcoming me, and for building a comfortable working environment: **Helmer** Belbo, **Kobra** Maleki, **Ari** Hietala, **Bless** Kufoalor, **Collins** Kukunda, Ghulam **Qasim** Khan and everyone else. I would also thank some former colleagues: **Lars** Bendik, **Wilson** Lara Henao (and his beautiful wife), **Saheba** Bhantagar, and **Joachim** Heppelmann. I would also like to thank the nice people I met at my department at NIBIO. Even though we did not meet often, those few times were always enjoyable.

I extend my gratitude to the team at SLU Umeå for their warm hospitality during all my stays there. Thank you, **Petra** Fant, **Juana** Chagas, **Brooke** Micke, **Johanna** Wallsten, **Sanna** Bergqvist, **Thomas** Eliasson, **David** Parsons, **Julien** More, **Julianne** Oliveira, **Gun** Bernes, and **Lotten** Markström. A special thanks to **Wondimagegne** Bekele for hours of discussion, making me discover Ethiopian food, and for trusting me so we could help each other out with our individual struggles!

I am profoundly grateful to my family for their unwavering support and for visiting me in Norway. **Marine** (and her **Quentin)** and **Lucas**, we had much fun with climbing the Seven sisters, fishing across Trøndelag, and watching puffins on Lovund. A special thanks to my **father** for all the support and love and helping me renovate a house while conducting a PhD at the same time. A special thanks to my **mother** for her total support, love, and guidance! I would also like to thank my cousin, **Olivier**, who dedicated his time to create the beautiful cover of this PhD book. A huge and special thanks to **Annabel** for her constant and precious support, companionship, and all the fun times we shared. I also wish to express my gratitude to **Garry** Slitherwell and **Moose** Miaould for their constant support. Thank you, **Manne** Bus, for forgiving me after a year, your wisdom is unmatched.

I am grateful for all the friends I made in Steinkjer from everywhere on earth (Nepal, Spain, Nigeria, Norway, Sweden, Belgium, England, Iran, India, Japan, Germany, Sri Lanka, Denmark, Czechia, etc.). A PhD abroad is not an easy journey, and it has become a lot easier to enjoy, thanks to your friendship. A special thanks to **Mie** Arnberg and **Maya** Nisha Situnayake, my two close friends and roommates. Thank you, **Maya**, for bringing me in Svalbard! A huge thanks to my "Gamle Mamas" who welcomed me and tried their best to teach me Norwegian. Finally, I'd like to thank all my friends in France. The fact that we can see each other once a year and feel like we haven't seen each other for a week is always amazing! Thank you also for visiting me in Norway and Steinkjer!

# An essential support during my PhD

The Less I Know The Better - Tame Impala Bitterzoet – Eefje de Visser This Old House Is All I Have – Against all Logic **MEGATRON** - Laylow Eye Nyam Nam 'A' Mensuro - Henrik Swarchz Blend Switched Up - Olivier Malcom the apathy song  $-$  maxime. Cat scratch - Dua Saleh Starry Night – Peggy Gou Contigo Jamas - Ocote Soul Sounds Red & Black Light (Live au Zenith Nantes Metropole, 2016) - Ibrahim Malouf Mo Yaro - Lass Jardin d'hiver - Henri Salvador I'm New Here – Gil Scott-Heron, Jamie xx Alpha Zulu - Phoenix CACHITO - Mitu, Loyal Lobos Xenon - Wallace Cleaver Tieduprightnow - Parcels Ivy – baby hayabusa, neophron Shawty U Know It Do - Yung Lean More Than a Love Song - Black Pumas Krunk – Sad Night Dynamite Utrus Horas - Orchestra Baobab Indi Groove - Chinese Man Giorgio by Moroder – Daft Punk Ascension – Pure Colors, Sould Food Horns Vem vet - Lisa Ekdahl Rose rouge – St Germain Pat Earrings - CASISDEAD Comme un Légo - Bashung Crystal – New Order Lovenote - Bladee I Won't Let You Down - Ph.D



 $\bf I$ 



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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

Quentin Lardy <sup>®a,b</sup>, Mohammad Ramin ®<sup>b</sup>, Vibeke Lind ®<sup>a</sup>, Grete Jørgensen ®<sup>a</sup>, Mats Höglind ®<sup>a</sup>, Emma Ternman  $\mathbf{C}^c$  and Mårten Hetta  $\mathbf{C}^b$ 

<sup>a</sup>Department of Grassland and Livestock, Norwegian Institute of Bioeconomy Research, NIBIO, Ås, Norway; <sup>b</sup>Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, SLU, Umeå, Sweden; 'Faculty of Biosciences and Aquaculture, Nord University, Steinkjer, Norway

#### **ABSTRACT**

This study compared animal performance and enteric methane  $(CH<sub>d</sub>)$  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<br>GreenFeed<sup>TM</sup> 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 CH<sub>4</sub> emissions. There was a rapid decrease in diurnal CH<sub>4</sub> 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  $CH<sub>4</sub>$  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 (CH<sub>4</sub>) 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  $CH<sub>4</sub>$  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 CH<sub>4</sub> production (g d<sup>-1</sup>) or CH<sub>4</sub> intensity (g kg milk−<sup>1</sup> ) compared with indoor feeding, and that the reduction in  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  emissions from grazing cows (Dall-Orsoletta et al., 2016; Cameron et al., 2018). Enteric  $CH<sub>4</sub>$  emissions from grazing dairy cows can be recorded using several techniques such as the sulphur hexafluoride  $(SF_6)$  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  $CH<sub>4</sub>$ emissions over the course of a day.

CONTACT Quentin Lardy quentin.lardy@nibio.no

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In PTG systems with both indoor feeding and grazing, it can be complicated to record  $CH<sub>4</sub>$  emissions over an extended period as the cows move between pasture and barn daily. As a result, the effects of each feeding environment on  $CH<sub>4</sub>$  emissions in a mixed feeding system are not fully known and a more accurate measuring approach is needed to map this relationship. The GreenFeed<sup>TM</sup> emissions monitoring (GEM) system (C-Lock Inc., Rapid City, SD) can record  $CH_4$  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  $CH_4$  emissions to be recorded. One GEM located on the pasture, and one installed in the barn enable the evaluation of PTG strategies to mitigate  $CH_4$  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  $CH<sub>4</sub>$  emissions from dairy cows in a PTG system at high latitudes. The  $CH<sub>4</sub>$  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 24 day (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<sup>-1</sup> d<sup>-1</sup> was provided in both treatments, which was three times the expected pasture intake (6 kg DM cow<sup>-1</sup> d<sup>-1</sup>) 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−<sup>1</sup> d−<sup>1</sup> ) 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  $m<sup>2</sup>$  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 Control™, 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 <sup>a</sup>	Silageb	Base concentrate <sup>c</sup>	Protein concentrate <sup>d</sup>
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

<sup>b</sup>Analysed by Eurofins (Food and Agri Sweden AB, Lidköping, Sweden).<br>SKomplett, Norm 180, tabulated values from manufacturer. (Lantmän Komplett Norm 180, tabulated values from manufacturer (Lantmännen<br>Lantbruk AB. Malmö. Sweden).

Lantbruk AB, Malmö, Sweden).<br><sup>d</sup>Addera Bas 350, tabulated values from the manufacturer (Lantmännen Lantbruk AB, Malmö, Sweden).

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<sup>-1</sup> silage, 490 g kg<sup>-1</sup> concentrate with 440 g kg<sup>-1</sup> of base concentrate (Komplett Norm 180, Lantmännen Lantbruk, Malmö, Sweden), 50 g kg<sup>-1</sup> 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−<sup>1</sup> ) 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

matter.<br><sup>a</sup>Calculated value based on proportion and feed value of each ingredient.

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

TDMI = 
$$
[(3.7 + \text{Parity} \times 5.7) + 0.305
$$
  
\n $\times$  MilkE (Mcal d<sup>-1</sup>) + 0.022  $\times$  BW (kg)  
\n $+ (-0.689 + \text{Parity} \times -1.87) \times \text{BSC}]$   
\n $\times [1 - (0.212 + \text{Parity} \times 0.136) \times e(-0.053 \times \text{DIM})]$  (1)

where TDMI is total dry matter intake (kg d<sup>-1</sup>), 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% ( $\pm$  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 2 bromo-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):

$$
ECM = MY(kg d^{-1}) \times [38.3 \times fat(g d^{-1}) + 24.2
$$
  
× protein(g d<sup>-1</sup>) + 16.54  
× lactose(g d<sup>-1</sup>) + 20.7]/3, 140 (2)

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

# 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  $CH_4$  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  $CO<sub>2</sub>$ , CH<sub>4</sub> and O<sub>2</sub>) and zero gas (N<sub>2</sub>). 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  $CO<sub>2</sub>$  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<sup>-1</sup>) 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<sub>4</sub> emissions (g d<sup>-1</sup>) were calculated as:

 $CH_4$ Combined GEM = [CH<sub>4</sub>Outdoor GEM(g d<sup>-1</sup>)  $\times$  visits Outdoor GEM + CH<sub>4</sub>Indoor GEM(g d<sup>-1</sup>) $\times$ visits Indoor GEM]/(visits Indoor GEM + visits Outdoor GEM)

(3)

where  $CH_4$  Combined/Outdoor/Indoor GEM is in g  $CH_4$ cow−<sup>1</sup> d−<sup>1</sup> and visits is number of validated visits to the GEM units (indoor and outdoor).

The  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  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  $CH_4$  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 (CH<sub>4</sub> and visits) from the two GEM units were used.

Figures (panel A) show the diurnal pattern of enteric  $CH<sub>4</sub>$  emissions plotted using arithmetic mean hourly CH<sub>4</sub> emissions values (g  $h^{-1}$ , 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 CH<sub>4</sub> 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,  $CH<sub>4</sub>$ , 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  $CH<sub>4</sub>$  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  $CH_4$  emissions recorded. The  $CH_4$ data were daily mean emissions per animal and GEM unit resulting in predicted daily  $CH<sub>4</sub>$  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 ( $p \leq 0.05$ ).

# 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<sup>-1</sup>), 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 CH<sub>4</sub> emissions (g h<sup>−1</sup>) per hour (7-day means of all validated CH<sub>4</sub> recordings, n = 292 for DAY), where horizontal lines indicate mean CH<sub>4</sub> 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 (CH<sub>4</sub> 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 (CH<sub>4</sub>) 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 CH<sub>4</sub> emissions (g h<sup>-1</sup>) per hour (7-day means of all validated CH4 recordings,  $n = 314$  for NIGHT), where horizontal lines indicate mean CH<sub>4</sub> 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 (CH<sub>4</sub> 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<sub>4</sub> emissions (g cow<sup>-1</sup> d<sup>-1</sup>) from the combined indoor and outdoor GEM units ( $p > 0.05$ ;  $d$  < 0.2). Moreover, CH<sub>4</sub> intensity (g CH<sub>4</sub> kg ECM<sup>-1</sup>) and CH<sub>4</sub> yield (g kg DMI<sup>-1</sup>) 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  $CH<sub>4</sub>$  value recorded in the outdoor GEM unit being lower (300 g CH<sub>4</sub> cow<sup>-1</sup> d<sup>-1</sup> or 12.5 g CH<sub>4</sub> cow<sup>-1</sup> h<sup>-1</sup>) than that recorded in the indoor GEM unit (414 g  $CH<sub>4</sub>$  $\text{row}^{-1} \text{ d}^{-1}$  or 17.2 g CH<sub>4</sub> cow<sup>-1</sup> h<sup>-1</sup>).

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  $(\pm$  SD).



Abbreviations: DM, dry matter; ME, metabolisable energy; CP, crude protein; NDF, neutral detergent fibre; OM, organic matter: SD, standard deviation. <sup>a</sup>Mean 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 Lindg-<br>ren (1979).

ren (1979).<br><sup>c</sup>Near-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  $CH<sub>4</sub>$  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).



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

mean.<br><sup>a</sup>Concentrate consumed in the concentrate feeder and the GEM units.

b<sub>Estimated based on TDMI (NASCEM, 2021)</sub> minus recorded intake indoors.<br>SEstimated from the NASCEM equation (2021) Estimated from the NASCEM equation (2021).

<sup>d</sup>Energy-corrected milk calculated as in Sjaunja et al. (1990).

<sup>e</sup>Milk analysis was performed on four consecutive samplings on the last two days of the recording period.

Table 4. <sup>E</sup>ffects 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.



Abbreviations: CH<sub>4</sub>, methane; ECM, energy-corrected milk; DMI, dry matter intake; GEM, GreenFeed emissions monitoring unit; SEM, standard error

of mean.<br><sup>a</sup>Combined GEM unit values are sum of {emissions value multiplied by number of visits per GEM unit}, divided by total number of visits.

 $b$ Using the CH<sub>4</sub> combined GEM for CH<sub>4</sub> intensity and yield. <sup>c</sup>Energy-corrected milk calculated as in Sjaunja et al. (1990).

<sup>d</sup>Where 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.

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<sup>-1</sup> h<sup>-1</sup>, respectively, and outdoor emissions were 12.1 and 12.6 g cow<sup>-1</sup> h<sup>-1</sup>, respectively. The effect size  $(d > 0.8)$  showed a difference in CH<sub>4</sub> 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

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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  $CH_4$  emissions were reduced at pasture for both DAY and NIGHT groups, indicating the potential of fresh grass inclusion in the diet to reduce  $CH_4$  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<sup>-1</sup> (Spörndly, 2003). The herbage grazed in the present

Table 5. <sup>E</sup>ffects 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	<b>NIGHT</b>	<b>SEM</b>	<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 <sup>a</sup>				
Grazing duration (h)	3.9	4.7	0.22	0.001
Grazing duration per access time <sup>b</sup> (%)	38.9	32.7	1.86	0.002
Grazing activity <sup>c</sup> (h)	1.1	1.4	0.08	0.004

Abbreviations: SEM, standard error of mean.

<sup>a</sup>When eating behaviour took place on pasture, it was interpreted as grazing

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  $\times$  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

behaviour.<br><sup>b</sup>Percentage 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<sup>-1</sup>. 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  $(d > 1)$ 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  $CH<sub>4</sub>$  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  $CH_4$  emissions,  $CH_4$  intensity (g kg  $\mathsf{ECM}^{-1}$ ) and  $\mathsf{CH}_4$  yield (g kg  $\mathsf{DMI}^{-1}$ ). Total  $\mathsf{CH}_4$ emissions (from combined GEM) were consistent with other European values (range 251–498 g  $d^{-1}$ , mean 376 g d<sup>-1</sup>) reported in a meta-analysis by Appuhamy et al. (2016). The  $CH_4$  emissions under PTG conditions have previously mostly been recorded using the sulphur hexafluoride tracer technique (SF $_6$ ) (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).

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Dairy cows in a previous PTG experiment, with similar TDMI and DMI proportions between indoor and pasture, produced more CH<sub>4</sub> (+109 g d<sup>-1</sup>; 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  $CH<sub>4</sub>$ measuring techniques (SF $_6$  vs GEM). Koning et al. (2022) investigated  $CH<sub>4</sub>$  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<sup>-1</sup> over the years which are similar to the values obtained in the present study.

The  $CH<sub>4</sub>$  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 CH<sub>4</sub> 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 CH<sub>4</sub> emissions were consistent with values found by Waghorn et al. (2016) for cows on pasture (10-15 g  $h^{-1}$ , 240-360 g d<sup>-1</sup>). 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  $CH_4$  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  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  emissions. More research is needed to identify the mechanisms by which fresh grass fermentation reduces  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  are also worth exploring. GEM units in a part-time grazing system

Recordings of  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  data on pasture, six cows never learned to visit the GEM unit frequently 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  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  emissions. However, there was a rapid shift in recorded  $CH<sub>4</sub>$  emissions between the indoor and outdoor settings, with  $CH<sub>4</sub>$ 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  $CH<sub>4</sub>$  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  $CH<sub>4</sub>$  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 Åkerlind 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.

## ORCID

Quentin Lardy D http://orcid.org/0000-0002-9963-8439 Mohammad Ramin http://orcid.org/0000-0002-0028-896X Vibeke Lind http://orcid.org/0000-0002-1857-3304 Grete Jørgensen <sup>1</sup>http://orcid.org/0000-0002-8533-5269 Mats Höglind **b** http://orcid.org/0000-0002-7474-8789 Emma Ternman Dhttp://orcid.org/0000-0001-6966-8621 Mårten Hetta http://orcid.org/0000-0002-8396-9786

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# Predicted methane production from Italian ryegrass pastures with contrasting chemical composition under sheep grazing in Northern Norway

Q. Lardy<sup>1,2</sup> | M. Ramin<sup>2</sup> | M. Hetta<sup>2</sup> | G. H. M. Jørgensen<sup>1</sup> | V. Lind<sup>1</sup>

1 Department of Grassland and Livestock, Norwegian Institute of Bioeconomy Research, Tjøtta, Norway

<sup>2</sup> Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Umeå, Sweden

#### **Correspondence**

V. Lind, Department of Grassland and Livestock, Norwegian Institute of Bioeconomy Research, Tjøtta, Norway. Email: vibeke.lind@nibio.no

#### Funding information

Norsk Institutt for Bioøkonomi, Grant/Award Number: 19/00310-14; Trondelag county authorities, Grant/Award Number: RT 21.05; Steinkjer Municipality, Grant/Award Number: 2019/198; Sveriges Lantbruksuniversitet, Grant/Award Number: 2020-02977

## Abstract

Background: The Norwegian sheep farming system relies on forages, such as grass silage during winter and grazing cultivated leys and rangeland pastures during summer. Sheep and other ruminants produce enteric methane  $(CH_4)$ , a greenhouse gas of interest, and there is a need for reliable data on gas emissions from sheep capturing both the indoor feeding period and the grazing season. This study utilized an in vitro gas technique (with standard cow rumen fluid) and modeling approach to estimate  $CH<sub>4</sub>$  production and fermentation patterns based on two different qualities of Italian ryegrass (Lolium multiflorum) pasture under sheep grazing.

Results: Herbage quality was examined for two 10-day periods, in July and August. Differences in chemical composition of the herbage during these periods had an impact on herbage digestibility and  $CH_4$  production. Total gas production and  $CH_4$  levels were significantly higher for lower quality herbage grazed in July than for higher quality herbage grazed in August ( $p < 0.005$ ). Production of volatile fatty acids in the rumen remained constant between the two periods, but the higher acetate to propionate (A/P) ratio correlated with the higher  $CH<sub>4</sub>$  production.

Conclusion: These findings suggest that pasture quality is an important factor to consider when implementing grazing strategies to reduce enteric  $CH_4$  production in sheep.

KEYWORDS grazing, in vitro, Lolium multiflorum, modeling, Norway

# INTRODUCTION

Grassland- or rangeland-based sheep farming systems worldwide are a more sustainable option than intensive livestock systems. $<sup>1</sup>$  The</sup> availability of land suitable for human-edible crops is limited, but sheep can contribute to food supplies without triggering feed-food competition.<sup>2</sup> In Norway, less than 3.5% of total land area is used for agriculture and around 50% of the agricultural land consists of permanent grasslands and meadows.<sup>3</sup> The sheep industry plays an important role in Norwegian agriculture due to the capacity of sheep to convert biomass from grassland into high-quality protein for human consumption. Ruminants are known to produce enteric methane  $(CH_4)$  when digesting their feed, however, there is an urgent need for reliable data on greenhouse gas (GHG) emissions from different types of livestock in their local environment, to provide accurate estimates of how emissions are affected by ruminant diets, during indoor feeding or grazing.

Norway is the largest sheep meat producer in the Nordic region, with 1.16 million sheep slaughtered in  $2022<sup>4</sup>$  The Norwegian sheep farming system relies heavily on forages, in the form of grass silage during winter and herbage from grazing for 5–6 months on cultivated

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leys during spring and autumn and on rangeland pastures during summer. In Norwegian studies by Lind et al.<sup>5</sup> and Åby et al.<sup>6</sup> enteric CH<sub>4</sub> emissions from sheep fed indoors were measured. Pasture quality and forage intake by grazing animals affect the  $CH_4$  emissions<sup>7</sup> but measurements from grazing sheep in Norway, or other Nordic countries, have not been carried out. There is a probability that the reported emissions from Norwegian sheep only are representative of the indoor season. Farmers need improved information about the annual GHG emissions by accounting for emissions from sheep on pasture to implement good mitigation strategies under arctic conditions with 24-h daylight during the summer.

In vitro studies can predict enteric  $CH<sub>4</sub>$  production in ruminants with reliable accuracy, $8,9$  and can help to identify promising strategies for later in vivo implementation while reducing experimental costs. It is therefore necessary to carry out direct measurements on pastures to obtain more accurate predictions of overall annual CH<sub>4</sub> emissions from sheep.

This study utilized an in vitro and modeling approach to estimate  $CH<sub>4</sub>$  production, digestibility, and fermentation patterns based on two different qualities of Italian ryegrass (Lolium multiflorum) pasture grazed by sheep. The hypothesis was that a high-quality pasture (less structural carbohydrate, higher crude protein, better digestibility, more energy/g dry matter [DM]) decreases  $CH_4$  production/kg DM consumed compared to a low-quality pasture.

## MATERIALS AND METHODS

Herbage sampling and a sheep grazing trial were conducted at the Norwegian Institute of Bioeconomy Research (NIBIO) station Tjøtta (65°49'58.3" N 12°25'46.5") in Northern Norway during summer 2020. The study plan was reviewed and approved by the Norwegian Food Safety Authority (FOTS 23005). An in vitro experiment on herbage samples was performed at the Swedish Agricultural University (SLU) in Umeå (Sweden), with handling of animals carried out with the permission of Swedish Ethical Committee on Animal Research (represented by the Court of Appeal for Northern Norrland), which approved the experimental protocol (permit no. A 32-16) in line with Swedish laws and regulations regarding EU Directive 2010/63/EU on animal research.

# Experimental design

#### Treatment, pastures, and animals

Herbage sampling was carried out in two 10-day recording periods, period 1 (July 16–25; low-quality pasture) and period 2 (August 11– 20; high-quality pasture). In July, the pasture was unfertilized and cut 10 days prior to the first herbage sampling, with a short regrowth period. After period 1, 20 kg/ha of mineral fertilizer (12-4-18 NPK, Felleskjøpet Agri) was applied and the pasture left to grow for 30 days until herbage collection started in period 2.

The pasture was a second-year Italian ryegrass ley dominated by L. multiflorum (91%), with 6% smooth meadowgrass (Poa pratensis) and

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3% other species such as white clover (Trifolium repens), meadow fescue (Festuca pratensis), and weeds. The experimental area of 2000  $m<sup>2</sup>$ was divided into four parallel blocks of 500  $m^2$  each. Each block was further split into five (1-5) subplots of 100 (10  $\times$  10 m) m<sup>2</sup> each, resulting in a total of 20 subplots. Herbage samples were collected from each subplot within blocks every second day.

After each sampling of a subplot, the available biomass in each of the four blocks was grazed by a flock of four nonpregnant Norwegian ewes. The 16 ewes were grouped based on initial live weight (66.5  $\pm$  16.8 kg) and age (1.9  $\pm$  1.6 year). Each group of ewes was allocated to the same block and over the 10-day period grazed all five subplots.

Prior to period 1, the ewes were adapted to the pasture for 1 month. Between period 1 and period 2, the ewes grazed a similar pasture nearby. The animals had access to shelter and water during the trial.

#### Pasture measurements and dry matter calculations

A quadrant (50  $\times$  50 cm) was randomly placed at three positions within each subplot. Compressed herbage height was recorded using a modified plate meter $10$  and then herbage mass was mechanically clipped (Bosch Iso cordless grass shears, Robert Bosch GmbH, Germany) at 3 cm above ground level. The herbage was weighed, dried (60°C for 72 h), and weighed to determine DM concentration (%). The dry herbage samples were milled (Retsch SM 2000, Retsch GmbH, Haan, Germany) to pass through a 1-mm screen and analyzed by near-infrared spectroscopy (NIRS;  $n = 120$ ) at the NIBIO laboratory in Særheim.<sup>11</sup>

After herbage sampling, each group of sheep was allowed to graze the subplots for 2 days. Dry matter intake (DMI) by the ewes during that period was estimated using the herbage disappearance method (HDM), based on the difference between herbage mass before and after grazing.<sup>12</sup> One regression per subplot for herbage mass was performed, based on the compressed sward height and DM content before grazing by the sheep. After 2 days of grazing per subplot, post-grazing herbage height was recorded at 100 points and estimated average daily DMI was calculated as the difference between the grass mass before and after grazing, divided by the number of days (2) and number of animals (4) per subplot.

# In vitro incubation

The dry herbage samples were pooled within subplots across blocks to one sample per 2-day grazing bout per period, resulting in five samples per period. In vitro incubations were performed to determine in vitro organic matter digestibility (IVOMD) and ruminal fluid digestible organic matter (VOS). Organic matter digestibility (OMD, %) and metabolizable energy (ME, MJ per kg OM) were calculated according to Lindgren.<sup>13</sup>

Two rumen-cannulated lactating Swedish Red cows fed ad libitum on a diet of 600 g/kg grass silage and 400 g/kg concentrate on a DM basis were used as donor animals of rumen inoculum for all incubations. The procedure, sampling, and measurements followed the

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protocol of Fant et al. $^{14}$  In short, rumen fluid from the cows was filtered and equal amounts from each cow were blended and buffered to a 1:4 ratio fluid: buffer by volume. Herbage DM substrate (1003  $\pm$  1.8 mg) was weighed into serum bottles, flushed with CO<sub>2</sub>, and 60 mL buffered rumen fluid (BRF) was added. All bottles were placed in a water bath and continuously agitated at  $39^{\circ}$ C for 48 h. The procedures were repeated for two runs with three replicates of each herbage sample in each run.

#### In vitro predicted methane production

Gas production was monitored using a Gas Production Recorder (GPR-2, Version 1.0 2015, Wageningen, UR), with readings every 12 min adjusted to normal air pressure (101.3 kPa). Measurement of CH4 production in vitro was performed following Ramin and Huhtanen.<sup>15</sup> In short, gas samples (0.2 mL) were withdrawn from each bottle at 2, 4, 8, 24, and 48 h during the incubation period and analyzed using a Varian Star 3400 CX gas chromatograph. Mean blank gas production within each run was subtracted from the sample gas production. Predicted  $CH_4$  production was calculated, and model simulations used a mean retention time of 50 h, corresponding to the maintenance level of feed intake in sheep as described by Ramin and Huhtanen.15

#### Volatile fatty acids

Liquid samples were extracted from the bottles after 48 h of incubation, with 0.6 mL liquid residue preserved at  $-20^{\circ}$ C. Volatile fatty acid (VFA) concentration was determined by ultra-performance liquid chromatography (UPLC; Waters Acquity), following the method of Puhakka et al.<sup>16</sup> Total VFA concentration (mmol/L) was calculated by mean blank VFA concentration. Total VFA production (mmol) was derived by multiplying the concentration difference (sample – blank) by the sample volume (60 mL). Molar proportion of individual VFA was related to total VFA.

#### Statistical analysis

All statistical analyses were performed using R software (R Core Team, 2021). Data on total gas and  $CH_4$  production parameters, total VFA production, and molar proportions of VFA were subjected to analysis by a mixed effect model, with sampling period (July and August) as a fixed effect and bottle, run and days as random effects. The chemical composition of herbages was analyzed as the mean of the three samples from each subplot using the above model without run and bottles. Outliers, 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 statistical analysis. Linear regressions between predicted  $CH<sub>4</sub>$  and chemical composition (mean per 2 days subplots) were performed to look for relationships. Differences were considered statistically significant at  $p \leq 0.05$ .

# RESULTS

The estimated DMI of the sheep was higher ( $p < 0.005$ ) in August (2.4 kg DM/animal/day) than in July (1.5 kg DM/animal/day). The chemical composition of herbage sampled in the two periods differed significantly for all parameters except potentially digestible NDF (pdNDF;  $p = 0.07$ ) water-soluble carbohydrates (WSC;  $p = 0.23$ ; Table 1). The herbages from August had significantly higher ME content ( $p < 0.005$ ) than those from July. Percentage NIRS digestibility and in vitro OM digestibility were similar within period (67% and 68%, respectively, for July; 77% and 76%, respectively, for August). Crude protein (CP) concentration was significantly higher for August herbages (+32%) compared with July herbages. The sward height before and after grazing was greater in August (27.5 and 8.8 cm, respectively) than in July (13.5 and 5.8 cm, respectively), but the herbage mass offered to the animals was similar ( $p > 0.05$ ; data not shown) due to the lower DM concentration of the grass in August (15%) compared to July (20%).

Estimated asymptotic in vitro gas production did not differ between the herbages from the two periods ( $p = 0.208$ ; Table 2). Total in vitro gas production  $(+8%)$  and fermentation rate  $(+19%)$  in July were significantly higher ( $p < 0.005$ ) than in August. Asymptotic CH<sub>4</sub> production (+10%) and predicted in vivo CH<sub>4</sub> production (+11%;





Abbreviations: ADF, acid-detergent fiber; CP, crude protein; DM, dry matter; iNDF, indigestible neutral detergent fiber; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy; NDF, neutral detergent fiber; OM, organic matter; pdNDF, potentially digestible NDF; SEM, standard error of the mean; WSC, water-soluble carbohydrate.
# PREDICTED IN VIVO METHANE PRODUCTION FROM GRAZING SHEEP **319 SECTION CONTINUES** SCL 319





Abbreviations: CH<sub>4</sub>, methane; DM, dry matter; SEM, standard error of the mean.

TABLE 3 Effects of harvesting herbage in July and August on in vitro total VFA production, VFA molar proportions, and VFA molar ratio at 48 h of incubation of buffered rumen fluid.



Abbreviations: A, acetate; DM, dry matter; P, propionate; SEM, standard error of the mean; VFA, volatile fatty acid.

mL/g DM) were higher for the herbage from July compared with the August herbages ( $p < 0.005$ ). The mean response of CH<sub>4</sub> production to increased iNDF concentration in the herbage was 0.22 g CH<sub>4</sub>/kg of DM per 1 g/kg of DM in iNDF. The WSC:CP ratio in July (2.03) was twice that in August (1.02). The mean response in  $CH_4$  production to CP was  $-0.52$  g CH<sub>4</sub>/kg of DM per 1 g/kg of DM in CP, with the highest adjusted  $R^2 = 0.54$  of all regressions tested (data not shown).

Total VFA and molar proportion of butyrate were similar  $(p = 0.458$  and 0.273, respectively) between periods (Table 3). There was a trend for July herbage for higher acetate molar proportion in the BRF ( $p = 0.066$ ) and a lower propionate molar proportion  $(p = 0.055)$  compared with August herbage. A significant difference in A/P ratio ( $p = 0.029$ ) was identified, with August having a lower ratio in BRF than July.

# **DISCUSSION**

Performance of in vivo studies is costly and labor demanding compared to the use of in vitro studies to screen the effect of different diets on  $CH_4$  production. Fant and Ramin<sup>9</sup> found a high correlation between CH<sub>4</sub> production between predicted in vivo and observed in vivo study. However, as pointed out by Yáñez-Ruiz et al.<sup>17</sup> testing diets in vitro do not guarantee a similar result when tested in vivo.

The present study was the first to use an in vitro approach to estimate  $CH<sub>4</sub>$  production and fermentation patterns under grazing sheep in Nordic conditions. The results contribute to develop best mitigation practices under arctic conditions with 24-h daylight during the grazing season.

# Herbage

Herbages harvested in the two periods differed for all chemical and nutritional parameters except concentrations of WSC. Therefore, the agronomic management regime applied (i.e., no application of chemical fertilizer and 10 days of regrowth in July vs. 20 kg/ha chemical fertilizer and 30 days of regrowth in August) successfully differentiated herbage quality between the two periods. The change of agronomic regime, increased herbage digestibility and CP concentration, maintained WSC concentration and decreased the concentrations of structural carbohydrates (NDF, iNDF, and ADF) in August compared with July. The difference in chemical composition between the two periods was greater for the ratio of nonstructural carbohydrate to iNDF. These differences might have been amplified by dry weather in the weeks preceding the July sampling.

Herbage ME content can be used to assess herbage quality.<sup>18</sup> The higher ME content of the August herbage (11.9 MJ/kg DM)

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compared to that from July (10.5 MJ/kg DM) indicated an overall better herbage quality offered in August. Herbage quality in the two periods was comparable to that of some forages described in the Nordic feeding table (Norfor), $19$  with August herbage corresponding to high-digestibility meadow (Norfor code: 006-0502) and July herbage to low-digestibility meadow (Norfor code: 006-0504). Due to the management regime applied, the results differed from those reported by Rivero et al.<sup>20</sup> who found that a longer regrowth period increased the concentration of WSC and decreased the concentrations of CP and NDF.

# Methane

In a study by Åby et al.<sup>6</sup> they recorded a DMI of 1.73 kg DM/animal/day for Norwegian White sheep while Lind et al. (pers com) found an average of 2.14 kg DM/animal/day. We did not establish a regression for the post-herbage mass, which likely resulted in overestimation of DMI due to higher stem bulk density and lower leaf bulk density, and thus DM density, in the lower half of the herbage. This effect might have been greater for August due to higher postherbage height after grazing.

According to Rinne et  $al.^{21}$  increased herbage digestibility enhances rumen fermentation and thus increases  $CH<sub>4</sub>$  production in ruminants. However, higher digestibility is associated with higher feed intake and passage rate, lower fiber content and as a result, a lower  $CH<sub>4</sub>$  production per kg DMI.<sup>22</sup> In the present study, the pasture in August had higher digestibility than in July but a lower digestible NDF content, which may explain the lower  $CH_4$  production estimated in August. The higher CP content in August herbage due to application of nitrogen fertilizer may also play a role. It is suggested by Jentsch et al.<sup>23</sup> that fermentation of CP produces less  $CH<sub>4</sub>$  than fermentation of carbohydrates. Lower  $CH_4$  production can therefore be attributable to replacement of carbohydrates by CP in the diet. $24$  This trend was confirmed by the negative slope of the regression between CP on CH<sub>4</sub> (-0.52 g CH<sub>4</sub>/kg of DM per 1 g/kg of DM in CP). It was even more apparent on comparing the WSC:CP ratio in the herbages, which was twice as great in July as in August. In vitro gas and  $CH_4$  emissions found by Sun et al.<sup>25</sup> for incubated ryegrass with DM, NDF, and ADF concentrations comparable to ours, were lower than those found in this study (35-36 mL CH<sub>4</sub>/g DM and 40-41 mL CH<sub>4</sub>/g DM, respectively). Their  $CH_4$ /total gas ratio was comparable (14.6%-14.9%) to that in the present study (14.8%–15.4%). The mean values obtained for seven different perennial ryegrass species incubated by Purcell et al.<sup>26</sup> with lower content of NDF, higher CP, and similar WSC concentrations as in the present study, were in a similar range (33.9– 35.1 mL CH $_4$ /g DM). The literature shows that our results are comparable when using the in vitro approach for predicted  $CH_4$  production from ryegrass.

On converting our results expressed as mL  $CH<sub>4</sub>/g$  DM into g  $CH<sub>4</sub>/kg DM$ , we obtained values of 26.6 and 23.6 g  $CH<sub>4</sub>/kg DM$  for July and August, respectively. Åby et al.<sup>6</sup> found average CH<sub>4</sub> emissions of  $16.1-25.2$  g CH<sub>4</sub>/kg DM for two Norwegian sheep breeds and two silage qualities (early and late). On using the National Research Institute for Agriculture, Food and the Environment (INRAE) formula<sup>27</sup> instead of our DMI estimate to calculate daily CH<sub>4</sub>, we obtained values of 1.6 and 1.7 kg DMI for July and August herbage, respectively. Daily  $CH_4$  production by our ewes was then 41.6 and 40.1 g/animal and day in the two periods. These results are comparable to findings by Åby et al.<sup>6</sup> of 40.2 g CH<sub>4</sub>/sheep and day at daily DMI of 1.73 kg DM.

Additionally, the predicted in vitro  $CH_4$  production in our experiment is similar to those measured in vivo on sheep fed ryegrass in New Zealand.<sup>25,28</sup> Warner et al.<sup>29</sup> found that increased digestibility reduced  $CH_4$  production per unit of digestible DM. A study cited by Hristov et al.<sup>30</sup> found that changes in chemical composition of feed accounted for 20% of the variation in  $CH_4$  emissions from sheep fed fresh ryegrass of different compositions, while feed intake accounted for 80% of the variation when using the respiration chamber technique.

These findings align with our own, suggesting that variations in  $CH<sub>4</sub>$  emissions were relatively little influenced (11%) by the chemical composition of the ryegrass herbages, which aligns with da Cunha et al.<sup>31</sup> who found that the sward structure (sward height and herbage mass) is more important for explaining the  $CH<sub>4</sub>$  emissions than the chemical content of a pasture.

## Volatile fatty acids

VEAs were extracted after 48 h of incubation. Hetta et al.  $32$  found an increase of acetate, propionate, and butyrate over time in in vitro recordings (7 measures over 96 h) but the A/P ratio remained unchanged in the BRF.

Several studies have found that decreasing forage digestibility and increasing fiber content influence total rumen VFA and molar proportions of VFA, with a greater acetate and lower propionate proportion.26,33–<sup>35</sup> Total VFA in BRFs in the present study was not influenced, but a tendency of acetate and propionate molar proportions was found, resulting in a lower A/P ratio in the August herbages. Lower A/P ratio means that a greater number of VFAs act as a net sink for hydrogen, reducing their capacity to form  $CH<sub>4</sub>$ . Rivero et al.<sup>20</sup> found similar results in experiments on autumn and spring standard ryegrass cultivar pasture with a A/P ratio of 3.08 and 2.67, respectively, but the  $CH_4$  output after 24 h of incubation (33.4 and 34.1 mL/g DM) was slightly lower than in our study. Purcell et al. $^{26}$  highlighted the lack of differences in rumen in vitro fermentation and the relative proportions of the major VFAs in BRF when the differences in composition (WSC and NDF) are small. We were able to accentuate these trends through agronomic management, which ensured that differences in chemical composition between harvesting periods were significant.

In this experiment, we used adult ewes. However, under the Norwegian sheep farming system, ewes and their lambs are gathered from the mountain summer pastures during August, and lambs not ready for slaughter (<40 kg live weight) are separated from their mothers and finished on Italian ryegrass. The pasture in August may cause higher feed intake of the animals resulting in higher absolute  $CH_4$ emissions. However, the weight gain of lambs on these pastures is similar to that of lambs fed a grass silage and concentrate diet<sup>36</sup> and the  $CH_4$  yield is likely to be lower with increasing pasture quality. Using pasture for fattening as a mitigating option must be considered regarding application of fertilizer and use of fuel against use of a diet consisting of grain-based concentrate and grass silage. The protein sources in the concentrate are in most cases imported protein, and the production of grass silage also needs fertilizer and fuel for machinery. Calculation of the emission intensity, g  $CH<sub>4</sub>/kg$  meat, is out of the scope of this experiment but is important to include in future research. To recommend farm practices for more sustainable production, not only GHG emissions from livestock but also the environment, yield, quality, and profitability of the entire system must be considered.

# CONCLUSIONS

Observed differences in chemical composition of ryegrass herbage during the season led to differences in in vitro  $CH_4$  production in sheep grazing. Herbages from July and August differed in qualities, leading to differences in predicted total gas and  $CH_4$  production. Although the VFA production remained constant during the rumen in vitro incubations, the molar proportions of individual VFA and A/P ratio showed differences that could explain the observed differences in predicted  $CH_4$  production. The overall conclusion is that high-quality herbage may reduce  $CH_4$  intensity in grazing sheep, so improving pasture quality is a tool to mitigate  $CH<sub>4</sub>$ emissions.

## ACKNOWLEDGMENTS

The authors wish to thank Thomas Karacs, Robert Sturite, and Thea Kongsmo for their help in Norway. We would like to thank all the staff at SLU in Umeå for their teaching and support in the in vitro incubation. Part of this work was an activity of the Grazing Living lab within the project SustAinimal (SLU, Umeå, Sweden).

## FUNDING INFORMATION

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

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

# ORCID

V. Lind https://orcid.org/0000-0002-1857-3304

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How to cite this article: Lardy Q, Ramin M, Hetta M, Jørgensen GHM, Lind V. Predicted methane production from Italian ryegrass pastures with contrasting chemical composition under sheep grazing in Northern Norway. JSFA Reports. 2024;4(8):316–22. https://doi.org/10.1002/jsf2.213

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Applied Animal Behaviour Science 282 (2025) 106477

Contents lists available at ScienceDirect



Applied Animal Behaviour Science



journal homepage: www.elsevier.com/locate/applanim

# Providing fresh pasture in the afternoon for full-time grazing dairy cows increases energy-corrected milk yield

E. Ternman <sup>a, 1</sup>, Q. Lardy <sup>b, c, 2</sup>, R. Danielsson <sup>c,\*,3</sup>, H. Gonda <sup>c, 4</sup>

<sup>a</sup> *Faculty of Bioscience and Aquaculture, Nord University, Steinkjer 7713, Norway*

<sup>b</sup> *Division of Food Production and Society, Norwegian Institute of Bioeconomy Research, Steinkjer 7713, Norway*

<sup>c</sup> *Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences (SLU), Uppsala 750 07, Sweden*



#### **1. Introduction**

High pasture intake during the grazing season is important for dairy production in general, and for organic dairy production in particular, as pasture usually makes up a substantial proportion of the forage ration. Well-managed pasture can be beneficial financially, as discussed by Wilkinson et al. (2020), and from an animal welfare perspective (Von Keyserlingk et al., 2017). However, it can be challenging to achieve a well-managed grazing system, since cows' motivation for seeking pasture varies (Charlton et al., 2013). One approach to overcome this is to take cows' diurnal behaviour into account and provide fresh pasture when the cows are most motivated to graze, thus promoting grazing behaviour. Several previous studies have found that grazing around dusk involves the longest and most intensive grazing events of the day (Gibb et al., 1998; Caram et al., 2021), and that allocation of new feed or pasture stimulates feeding behaviour and grazing activity (DeVries et al., 2003; Verdon et al., 2018). A study by Pollock et al. (2022) investigating pasture allocation frequency found that for multiparous cows, proving fresh herbage every 36 hours significantly increased their grazing activity compared with providing fresh herbage every 12 or 24 hours. Irrespective of pasture allocation frequency, cows in that study displayed a very distinct grazing pattern over the 24-hour period, with a

\* Corresponding author.

<sup>2</sup> 0000–0002-9963–8439

#### https://doi.org/10.1016/j.applanim.2024.106477

Received 2 July 2024; Received in revised form 2 December 2024; Accepted 10 December 2024

Available online 11 December 2024

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*E-mail address:* rebecca.danielsson@slu.se (R. Danielsson). 1 <sup>0000</sup>–0001-6966–<sup>8621</sup>

<sup>3</sup> 0000–0001-7244–061X

<sup>4</sup> 0000–0002-0452–8289

major grazing peak after afternoon milking and a smaller peak after morning milking (Pollock et al., 2022), demonstrating the benefits of providing fresh herbage in the afternoon. Rumen fill may also affect grazing behaviour and serve as a meal-eating regulator. In an extensive review, Gregorini et al. (2008) assessed the influence of pasture herbage composition, i.e. total intake in combination with particle reduction size of the herbage, on rumen fill. Different herbage species undergo different particle size reductions and thereby differ in their impact on rumen fill. Conventional pasture in the Nordic region consists of a combination of grass and legumes, of which grass has a slower particle size reduction than legumes (review by Gregorini et al., 2008). An increase in milk yield and in fat and protein yield has been observed for cows with access to pasture in the afternoon compared with the morning, which is suggested to be due to a change in nutrient content of the pasture over the day (Orr et al., 2001; Gregorini et al., 2008; Vibart et al., 2017). In short, the DM and water-soluble carbohydrate (WSC) content increase during the day, while the content of structural carbohydrates and crude protein decreases due to a dilution effect. This suggests that the nutritional value of herbage is more favourable during afternoon compared with morning grazing, due to a better-balanced ratio between fermentable carbon and nitrogen. Feed rations with a balanced carbon:nitrogen ratio can improve intake, milk yield and nitrogen use efficiency (Cosgrove et al., 2007; Pozo et al., 2022). We hypothesize that offering to lactating dairy cows fresh pasture in the afternoon-early evening, rather than in the morning, will result in an increase milk yield. The present study aimed to study changes in grazing behaviour, milk yield and composition, and dietary nitrogen utilization when dairy cows were allowed to access their daily grazing strip either after the morning milking or after the afternoon milking on an organic dairy farm in southwest Sweden.

#### **2. Material and methods**

The grazing experiment was carried out from 5 May to 28 May 2022 on an organic farm with a herd of 98 lactating dairy cows in the Halland region, southwest Sweden. The farm was chosen for being a successful low-input pasture-based dairy farm, non-common among Swedish dairy farms. According to the Swedish Animal Welfare act (SFS: 2018:1192) animals on private farms can be enrolled in research studies without an ethical permission required if treatments are part of their normal daily routines and if there is no invasive handling of the animals included. As the trial complied with these regulations, no ethical approval was required for this study.

#### *2.1. Animals and experimental design*

Sixty dairy cows were allocated to two treatment groups that were given access to a new strip of pasture after morning milking (treatment AM;  $n = 30$ ) or after afternoon milking (treatment PM;  $n = 30$ ). A period of pre-experimental recording started 10 days before starting the experiment and was used to record different metrics: 4 days of grazing behaviour, body weight (BW) and milk yield (MY) on two occasions, and animal information such as days in milk (DIM) and parity. This data was later used for grouping the cows into 2 homogenous groups and ensuring that no differences existed before start of the experiment in grazing behaviour between groups. Grazing time for cows later grouped into the AM group was 444  $\pm$  62.8 min and grazing time for cows grouped into the PM group was  $456 \pm 67.5$  min (P = 0.408), and milk yield was 29.0  $\pm$  4.78 kg and 30.3  $\pm$  5.09 kg, respectively (P = 0.355). The experimental cows were paired according to DIM, parity and milk yield, and within pair, randomly assigned to a treatment. For AM and PM cows, respectively, this resulted on average ( $\pm$  standard deviation, SD) in: body weight (BW)  $637 \pm 76.1$  and  $596 \pm 134.5$  kg; DIM  $153 \pm 58.3$ and 155  $\pm$  56.0; parity 1.6  $\pm$  0.50 and 1.5  $\pm$  0.50; and daily milk yield (MY) 29.0  $\pm$  5.0 and 29.6  $\pm$  5.4 kg. The remaining cows in the herd were allocated to either treatment, to create evenly distributed groups. In total, the experiment was run for three weeks of which the first two weeks were used to allow the cows to adapt to the new groups and to the grazing management system, and the last five days were used for data collection and sampling. Both groups spent all day on pasture except during milking. Milking took place in a  $2 \times 10$  swing-over milking parlour (SAC, S.A. Christensen and Co. Ltd., Kolding, Denmark), starting at 0500 h and 1430 h each day, and cows went back to pasture straight after milking. The groups were always milked in the order PM followed by AM. The milking break from fetch until return to pasture took approximately 1.5–2 hours. Each group of cows was offered strip grazing with herbage allowance *>* 40 kg DM per cow per day (according to grazing management on the farm), on plots of average size  $7500 \text{ m}^2$ . In addition to pasture, all animals were offered a grain-mix (50 % barley, 25 % wheat, 15 % rye, 10 % oats) of 2 kg during each milking, i.e. in total 4 kg grain-mix per day. Nutrient composition of the pasture and grain-mix are presented in Table 1. Minerals were included in the grainmix (Deltamin Bas Normal, Svenska foder, Lidköping, Sweden).

#### *2.2. Grazing management and pasture characteristics*

All cows were allowed one week of full-time grazing before the adaptation period began. Cows grazed on several pasture plots, following the farmer's normal routine, in a daily strip grazing system using temporary electric fencing. All cows had access to water on pasture throughout the experiment. The pasture used was established in 2021 using a seed mixture comprising 30 % perennial ryegrass (*Lolium perenne L*.), 26 % timothy (*Phleum pratense L.*), 17 % meadow fescue (*Festuca pratensis L.*), 13 % white clover (*Trifolium repens*), 6 % chicory (C*ichorium intybus*), 4 % plantago (*Plantago lanceolata*) and 4 % cumin (*Cuminum cyminum*). A new daily grazing strip was opened after milking, in morning or afternoon according to the treatment. In order to minimise differences between the daily grazing strips, cows in the two groups were offered bordering plots. The distance between the barn (milking parlour) and the grazing paddocks was approximately 1 km. Cows were fetched for milking morning and evening and herded back to the pasture after each milking.

Pre-grazing herbage mass was measured daily using a rising plate meter (Jenquip, Feilding, New Zealand; range 0–26 cm, plate area 0.1  $m^2$ ; weight 316 g). A total of 100 compressed heights were recorded while walking the pasture in a zig-zag pattern. To calculate the regression model of herbage mass (kg DM/ha; *dependent variable*) as a function of compressed sward height (cm; *independent variable*), herbage mass in 30 squares of 0.16  $m<sup>2</sup>$  was measured with a Jenquip rising plate meter and the sward was immediately cut as close to ground level as possible (approx. 1–3 cm stubble height) with electric clippers (Bosch Iso Cordless Grass shears, Robert Bosch GmbH, Germany). The cut herbage samples were dried to constant weight at 60 °C. Sward surface height  $(SSH; n = 50)$  was recorded before and after grazing, using a sward stick similar in design of the HFRO sward stick (Barthram, 1984), but in which the contact area measured 15 mm x 35 mm.

**Table 1** 

Nutritional content of hand plucked pasture samples  $(n = 10)$  and grain samples  $(n = 2)$ , mean (SD) offered to lactating dairy cows receiving fresh pasture either after morning or afternoon milking.

Feed chemical composition	Pasture	Grain
DM, $g/kg$	163 (17.1)	880 (0.88)
ME, MJ/kg DM	12.1(0.48)	۰
Ash, g/kg DM	84 (6.6)	34(4.4)
$CP$ , $g/kg$ DM	148 (24.1)	106(0.3)
WSC, g/kg DM	131 (24.8)	٠
Starch, g/kg DM	٠	553 (2.0)
aNDF, g/kg DM	323.2 (33.1)	154 (0.4)

Abbreviations: DM: Dry matter; ME: metabolisable energy; CP: crude protein; WSC: water-soluble carbohydrates; aNDF: amylase neutral detergent fibre.

#### *2.3. Botanical composition*

Immediately before cows accessed the new grazing strip, the botanical composition of the sward was determined as described by Mannetje and Haydock (1963), but by taking 30 images per strip from a height of approximately 1 m using a mobile phone camera (3024 x 4032 pixels), while walking the plots in a zig-zag pattern. The images were then analysed visually in a procedure where each image was divided into 12 equally sized squares, within which the areal coverage of five classes of species (grasses, plantago, clover, chicory, and other –composed by cumin and weeds-) was ranked on an arbitrary scale of 1–5, with 1 being the most dominant plant species per square. A value of zero was used when a group of species was not present. The most frequently occurring number per rank and species was found by using the MODE function in Microsoft Excel (Microsoft® Excel® for Microsoft 365 MSO, Version 2308 Build 16.0.16731.20052).

#### *2.4. Feed samples and analysis*

During the five days of the sampling period, herbage was sampled immediately before the cows accessed their new strip, in the morning for the AM group and in the afternoon for the PM group. Samples of pasture were hand-picked at 30 sites while walking a zig-zag transect, pooled and dried at 60 ◦C to constant weight, ground to pass through a 1-mm Wiley mill sieve and stored at room temperature prior to chemical analysis. To evaluate diurnal variation in chemical composition, samples of grasses, plantago, chicory, and white clover were hand-picked separately at 0700 and 1700 h on all five days of the sampling period. These samples were immediately frozen in the field by submersion in liquid nitrogen (N), and then dried at 60 ◦C to constant weight, ground to pass through a 1-mm Wiley mill sieve and kept at room temperature until analysis. The grain-mix offered to the animals at milking was sampled on two occasions during the sampling period and the samples were stored in plastic bags in a dry place for later analysis.

Feed analyses were performed by the laboratory at the Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Uppsala, Sweden. The hand-plucked pasture samples and grain-mix samples were analysed by conventional chemical analyses, using standard methods for determination of DM, crude protein, neutral detergent fibre (NDF, assayed with a heat-stable amylase and expressed exclusive of residual ash; Chai and Udén, 1998), WSC, ash, *in vitro* organic matter digestibility (VOS) (from which metabolisable energy (ME) was calculated), as described by Bertilsson and Murphy (2003) and Volden (2011). Starch (including maltodextrin) in concentrate samples was analysed enzymatically according to Larsson and Bengtsson (1983). CP was analysed using the automated Kjeldahl procedure (Foss, Hillerød, Denmark).

### *2.5. Animal measurements*

Milk yield of all experimental cows was recorded manually using a Tru-Test sampler (Tru-Test Datamars, Auckland, New Zealand) at each milking during the sampling period. Samples for milk composition were collected during the last four milkings of the sampling period, preserved with bronopol and then refrigerated. Body weight of each animal was recorded using a portable cattle scale after morning milking on the first two days of the study period and on the last two days of the sampling period. For the behaviour data, 53 cows (AM  $n = 26$ , PM  $n = 27$ ) were equipped with Nedap SmartTag neck sensors (Nedap Livestock Management, DC Groenlo, The Netherlands), which automatically recorded four different behavioural states (eating during grazing, ruminating, idling and other) (Rue et al., 2020). For one cow (PM), only eating during grazing was recorded by the sensor. The behaviour information was obtained in datasets containing observations for each cow at 1-min intervals. For ease of reading, the behaviour "eating during grazing" is referred to hereafter simply as "grazing".

Data in one-minute bins were summarised within experimental days for each cow. If one cow lacked more than 10 % data points per day, all values for that day were set as "missing". One cow from the AM treatment was eliminated from the dataset, due to missing more than two full days of data (2880 data points or minutes). Outliers for each behaviour were identified in the dataset for experimental days and removed according to the  $\pm$  1.5 inter-quarter range (IQR) method. Before statistical analysis, data for each experimental cow day were averaged over the whole sampling period. Hourly durations of grazing, ruminating, and idling were computed using the arithmetic mean, and averaged over the day (24 h) using data from the whole sampling period.

#### *2.6. Animal behaviour*

Event duration for grazing and ruminating was calculated for group comparison only, by extracting stop and start times for each event and calculating the difference. Events were counted as unique events independent of length of time in between. For example, if a cow was recorded as ruminating for 24 minutes, interrupted by idling behaviour for one minute and then back to ruminating, this was counted as two separate ruminating events. The duration of each event, the maximum duration of events and the number of events were calculated per day and averaged over the sampling period. Events were defined as belonging to one experimental day depending on start time and were allowed to continue over a day shift. Grazing data were also aggregated into 2, 4 and 6 h post-milking for each group, in an attempt to separate the effect of fresh pasture from simply returning from milking.

### *2.7. Weather data*

Outdoor temperature (C◦), precipitation (mm), wind speed (m/s) and relative humidity (RH, proportion) were recorded 2 m above ground level every 15 min by a weather station located in close proximity to the grazing area (*<*1 km). The data were aggregated into hourly mean, min and max before being transmitted to cloud-based data storage (Lantmet, VPE/SLU Fältforsk, Sweden), and later downloaded for further analysis (https://www.ffe.slu.se/lm/LMHome.cfm, Lantmet, VPE/SLU Faltforsk, ¨ Sweden, 1 June 2022).

Temperature humidity index (THI) was calculated as (NOAA, 1976):

$$
THI = F^{\circ} - (0.55 \quad (1 - RH)) * \quad (F^{\circ} - 58)
$$

In order to use this equation for calculation on THI, temperature measurements in Celsius were converted to Fahrenheit (F◦) according to:

$$
F^\circ = C^\circ \times \frac{9}{5} + 32
$$

Temperature, THI and precipitation were averaged for daytime (0700–1700 h) and night-time (1800–0600 h) for the whole sampling period.

#### *2.8. Herbage intake*

With the assumption that all cows consumed the 2 kg grain-mix offered at each milking, DMI was estimated by the NASEM (National Academies of Sciences, Engineering, and Medicine, 2021) and NEL20 (Norfor, 2011) approaches. NEL20 provides a feed value for net energy standardised at 20 kg of DMI. Since the cows in this study likely consumed close to 20 kg DM, we used NEL20 to estimate herbage intake. The NorFor model was used to estimate NEL20 of the feeds, and the animals' intake capacity and energy requirement (NorFor version 6.34, FST revision 2.10, FRC revision 2.15). All models were compared based on outcome for rumen fill and supply of energy requirement for each cow (data not shown). After this comparison, NASEM was chosen as being the best performing, as the model resulted in close to 100 % of intake capacity and energy requirement according to NorFor (2011), where mean rumen fill was  $103 \pm 3.7$  % of intake capacity and energy

supply was  $101 \pm 6.6$  % of energy requirement. The NASEM equation takes the form:

$$
DMI\left(\frac{kg}{d}\right) = \left[ ((3.7 + parity * 5.7) + 0.305 * MilkE + 0.022 * BW + (-0.689 + parity * -1.87) * BSC \right] \times [1 - (0.212 + parity * 0.136) * e^{(-0.053 + DM)}]
$$

where DMI is estimated dry matter intake in kg per day, BSC is body condition score (set to 3.5 for all cows), parity was set to 0 for primiparous cows and 1 for multiparous cows, BW is body weight of the cow in kg, MilkE is the energy needed for daily milk production in Megacalories (Mcal; 1 litre of milk requiring 1.39 Mcal) and DIM is the number of days in milk since last calving.

#### *2.9. Milk composition*

Milk samples were analysed using MIR spectroscopy (CombiScope FTIR 300HP, Delta Instruments B. V., Drachten, the Netherlands) for milk fat, protein, lactose, total solids and milk urea nitrogen (MUN), calculated according to Delta Instruments (2007).

Milk constituent concentrations were calculated as a weighted mean of the combined afternoon and morning milk yields. Daily ECM yield was calculated according to Sjaunja et al. (1990).

#### *2.10. Statistical analysis*

All data handling and figure design prior to statistical analysis were conducted using the R software (R 265 Core Team., 2021), unless specifically stated otherwise.Statistics were computed on the sampling period mean per cow for productive variables and behaviour. All variables were checked, and criteria met, for normality through the Shapiro-Wilks test using the univariate procedure (SAS 9.4 2016; Cary, NC, USA) in addition to visual inspection of the QQ plots. Homogeneity was checked through visual inspection of the residual plots. The effects of the treatments on behaviour, feed intake, body weight change, milk yield and milk composition were analysed in a generalized linear mixed model (SAS 9.4 2016; Cary, NC, USA). Variables included in the model as fixed effects were treatment (AM and PM; class variable), parity (primiparous and multiparous; class variable), DIM (continuous variable) and the interaction of treatment x parity. Pre-experimental milk yield was used as a covariate in the analyses for milk yield. The number of degrees of freedom was estimated by the Kenward-Roger approximation procedure. Unless otherwise stated, the values presented are least square means (LSM  $\pm$  SE). Differences between treatments were considered significant at  $P\leq 0.05.$ 

#### **3. Results**

#### *3.1. Weather conditions*

Mean  $( \pm SD)$  temperature, THI and precipitation for the region and the whole study period was  $11.3 \pm 3.74$  °C,  $52.6 \pm 5.92$  and 0.1  $\pm$  0.49 mm, respectively, and for the sampling period 11.0  $\pm$  2.95 °C,  $52.1\pm5.07$  and  $0.1\pm0.45$  mm, respectively. Temperature and THI at night (1800–0600h) during the sampling period was  $9.3 \pm 2.58$  °C and 49.3  $\pm$  4.62, respectively, while temperature and THI during the day (0700–1700 h) was  $12.9 \pm 2.05$  °C and  $55.4 \pm 3.30$ , respectively. During the sampling week, the sun rose at 0430 h and set at 2140 h, dawn occurred at around 0330 h and dusk at around 2240 h. At this latitude and time of the year there is only civil and nautical twilight, with no true darkness (astronomical twilight), during the night.

## *3.2. Pasture and feed quality characteristics*

Pasture characteristics and chemical composition of the herbage

were similar between the two treatments (Table 2). Herbage mass (on average, 2860 kg DM/ha), pre- and post- grazing SSH (on average 25.6 and 11.2 cm, respectively), as well as herbage allowance (on average, 64.7 kg DM/cow/d) were almost identical in the two treatments. The ME content per kg DM in the offered strips was also very similar for the AM and PM pasture (Table 2). The CP:WSC ratio in hand plucked samples, as well as in samples of plantago and chicory were similar between the strips (Table 2). The CP:WSC ratio was, however, higher in AM than in PM grass ( $P = 0.031$ ;  $F_{1.5} = 12.5$ ), and lower in AM than in PM clover ( $P = 0.012$ ;  $F_{1,5} = 21.4$ ). The botanical composition did not differ between the two treatments for all the species ( $P \ge 0.05$ ), except for chicory which its proportion was higher ( $P = 0.006$ ;  $F_{1,3} = 322$ ) in AM than in PM treatment (Table 2). Grass and plantago were the most common species in both treatments. Other species, comprising cumin and weeds, represented less than 1 % of the total dry herbage mass (Table 2).

#### *3.3. Behaviour, intake and body weight*

Daily duration of grazing was higher (P < 0.001; F<sub>1,48</sub>=14.0) and daily duration of rumination was lower ( $P < 0.001$ ;  $F_{1,47}=14.7$ ) in the PM group of cows compared with the AM group. There was no difference in idling time between cows in the two treatments  $(P = 0.13;$  $F_{1,47}=2.42$ ) (Fig. 1).

Irrespective of treatment, grazing and rumination both showed peaks in activity after milkings and at dusk (Fig. 2). There was a clear shift in behaviour around dawn, with cows switching from rumination to grazing (Fig. 2). The PM group spent numerically more time grazing (min/h) around dusk, while grazing by the AM group was more evenly distributed throughout the day (Fig. 2). Cows in the AM group spent slightly more time (min/h) ruminating during the night compared with cows in the PM group (Fig. 2).

There was a significant difference in grazing time (min/h) between the two treatments for each time interval studied (2, 4 and 6 h), where

#### **Table 2**

Pasture characteristics, and chemical and botanical composition of herbage in the strips offered to cows in the two treatments groups: access to new strip after morning milking (AM) or after afternoon milking (PM).

	AM	<b>PM</b>	<b>SEM</b>	p. value			
Pasture characteristics							
Pre-grazing surface height, cm	25.1	26.1	1.83	0.553			
Post-grazing surface height, cm	11.4	10.9	1.65	0.460			
Herbage mass, kg DM/ha	2802	2918	194.5	0.539			
Herbage allowance per strip, kg DM/	64.5	64.8	5.10	0.787			
cow							
Herbage							
DM, g/kg	158	167	9.52	0.333			
Crude protein, g/kg DM	162	164	13.2	0.900			
WSC, g/kg DM	142	146	12.8	0.990			
aNDF, g/kg DM	369	338	18.2	0.276			
Ash, g/kg DM	90.3	95.4	4.30	0.112			
ME, MJ/kg DM	12.0	12.2	0.29	0.305			
CP:WSC ratio							
Hand plucking	0.91	0.93	0.130	0.799			
Grass	1.85	1.48	0.523	0.031			
Plantago	0.71	0.83	0.076	0.717			
Clover	0.36	0.38	0.027	0.012			
Chicory	0.89	1.09	0.210	0.562			
Botanical composition (% Dry weight)							
Grass	38	31	7.04	0.181			
Plantago	25.2	31.6	6.12	0.107			
Clover	20.7	12.4	3.78	0.676			
Chicory	14.2	13.1	2.67	0.006			
Others	0.13	0.74	0.50	0.063			

Abbreviations: Abbreviation: SEM: standard error of means. DM: Dry matter; ME: metabolisable energy; CP: crude protein; WSC: water-soluble carbohydrates; aNDF: amylase neutral detergent fibre.

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**Fig. 1.** Average time spent idling, ruminating and grazing by lactating dairy cows in the two different treatments, access to new pasture after morning milking (AM)<br>or access to new pasture after afternoon milking (PM). P



**Fig. 2.** Diurnal behaviour pattern of lactating dairy cows as percentage of minutes spent per activity in the treatments (A) access to new pasture after morning milking (AM) and (B) access to new pasture after afternoon milking (PM).

the cows receiving fresh pasture spent more time grazing than the cows which were let out on old pasture (Fig. 3). There was also a numerical increased cumulative effect for the PM cows, which after 6 h on fresh pasture had spent 228 min grazing, compared with the AM cows, which only spent 146 min out of 6 h grazing on fresh pasture (Fig. 3). Estimated pasture intake and body weight did not differ between the treatments.



**Fig. 3.** Mean, standard error, F-value and statistical significance (\**<*0.05 and \*\*\**<*0.001) of difference in minutes spent on eating during grazing in the 2, 4 and 6 hours after morning and evening milking for lactating dairy cows receiving new pasture after morning milking (AM) or after afternoon milking (PM).

#### *3.4. Milk yield and composition*

Allocation time of a new strip did not affect milk yield (kg/d;  $P = 0.10$ ;  $F_{1,53} = 2.74$ ) or milk composition. However, cows in the PM group had higher ECM ( $P < 0.01$ ;  $F_{1.55} = 7.24$ ) and milk protein yield  $(P < 0.05; F<sub>1.55</sub>=5.80)$  than cows in the AM group (Table 4). Milk fat  $(P = 0.08; F<sub>1,55</sub>=3.15)$  and lactose  $(P = 0.09; F<sub>1,55</sub>=3.02)$  production per day tended to be higher in the PM group than the AM group, but concentration of MUN tended ( $P = 0.08$ ;  $F_{1,55} = 3.24$ ) to be lower in the PM group than the AM group. There was an effect of DIM on all variables except estimated DMI, milk protein yield per day and urea in milk (Table 4). The net energy for lactation requirement for the two treatments was 128 MJ/cow/day for the AM and 138 MJ/cow/day for the PM.

### **4. Discussion**

This study was conducted on a commercial organic dairy farm characterised as a low-input production system in which the aim of the farmer is to optimise use of pasture as the main feed resource during the grazing season. It is worth noticing, that the grazing routine used was daily strip grazing with relatively high forage allowance, and therefore results of this study shouldn't be generalized to different grazing managements. Weather conditions during the sampling period can be considered to lie within the temperature-neutral zone for lactating dairy cows (reviewed by Kadzere et al., 2002), with mean THI of 52.3. During the experiment, the farmer made all decisions on grazing management, e.g. pastures to be grazed and forage allowance. The only pre-set conditions were that both groups of cows should graze on similar pastures, which was fulfilled by allowing both groups of cows to graze neighbouring strips of the same cultivated ley throughout the study. Thus, as intended, pasture characteristics such as herbage mass, botanical and chemical composition of the herbage and SSH did not differ between the treatments (Table 2). In relation to the second pre-set condition, the herbage mass and allowance, herbage ME content, and the pre- and post-grazing surface heights suggest that the offered pasture did not limit herbage and energy intake in either of the treatments (Johansen and Höglind, 2007; Perez-Prieto and Delagarde, 2012; Mezzalira et al., 2014; Kunrath et al., 2020). The total grazing time recorded (Table 3) also supports the assumption that there were no restrictions on herbage

#### **Table 3**

Effect of time of access to new pasture, after morning milking (AM) or after afternoon milking (PM), on grazing, ruminating and idling behaviour in lactating dairy cows.



Abbreviation: SEM: standard error of mean; trt: treatment; DIM: days in milk.

intake. According to Pérez-Prieto and Delagarde (2012), average daily grazing duration under strip or rotational grazing management typically lies within the range 450–550 min/d, indicating that the cows in our study were motivated to graze as both groups were closer to the higher end of that range. However, in a study by Wales at el. (1999) herbage intake in lactating dairy cows increased linearly without reaching a plateau as the herbage allowance increased from 20 to 70 kg DM per cow and day on pasture of ryegrass and white clover, and as the herbage allowance increased from 25 to 50 kg DM per cow and day on pasture dominated by paspalum. The pre-grazing SSH of the pastures used by Wales et al. (1999) was rather low (7–9 cm), which may have been a limiting factor for high intake rate at any allowance quantity, as discussed by Mezzalira et al. (2014).

The most significant findings in the present study were the observed

increased grazing time, reduction in rumination time and increased ECM yield when cows were allowed to access the fresh grazing daily strip after afternoon milking (PM group) rather than after morning milking (AM group) (Tables 3 and 4). The increased grazing time in PM cows (Table 3) differ to the findings by Vibart et al. (2017), who in a similar experimental setting did not observe any differences in grazing time when late-lactation dairy cows were allowed fresh ryegrass-based pasture either after the morning or afternoon milking. It is worth noting that in the present study and in the study by Vibart et al. (2017), grazing time refers to eating time during grazing, i.e. it does not include other activities such as searching for feed during grazing. While there is no apparent explanation for the conflicting results obtained in the studies, the more prolonged grazing time in PM cows may be a consequence of cows displaying more intense grazing during dusk, as part of their natural diurnal rhythm, as shown in other studies (Gibb et al., 1998; Taweel et al., 2004; Gregorini et al., 2008; Kismul et al., 2019). Changes in chemical composition of the herbage during the day, i.e. increased content of WSC owing to ongoing photosynthesis resulting in increased digestibility and higher WSC:CP ratio in late afternoon-early evening, may be a motivation for more intense grazing around dusk (Provenza et al., 1998; Taweel et al., 2004; Gregorini et al., 2007). However, in our study, WSC and CP contents, as well as CP:WSC ratio did not follow a clear pattern, as seen by others (Delagarde et al., 2000; Orr et al., 2001; Gregorini et al., 2008; Vibart et al., 2017).

In ruminants, the main eating bouts are concentrated during the day and the main rumination bouts during the night (Rook and Huckle, 1997). Gibb et al. (1998) observed peaks in grazing behaviour at sunrise and sunset, but also the occurrence of multiple smaller meals between sunrise and evening milking, interspersed with intervals of ruminating and resting. In line with that, and irrespective of treatment, cows in our study displayed most of their grazing events during daytime (Fig. 2), as also observed by Iqbal et al. (2023). In addition, the grazing pattern displayed in our study was affected by both milking routine and the time of allocation of a new daily grazing strip, with a greater cumulative effect on grazing duration for cows receiving fresh pasture in the afternoon (Fig. 3). This demonstrates the positive effect of offering fresh pasture, independent of time of day, on cows' motivation to graze, and

#### **Table 4**

Effect of time of access to new pasture, after morning milking (AM) or after afternoon milking (PM), on milk production, milk composition, milk urea and body weight change in lactating dairy cows.

	AM	PM	<b>SEM</b>	р. value	P-value trt*parity	p-value <b>DIM</b>		
Animal metrics								
Estimated DMI,	20.1	20.4	0.43	0.581	0.923	0.710		
kg DM/day								
Milk yield, kg/	25.8	27.7	0.62	0.104	0.638	< 0.001		
day								
ECM, kg/day	26.0	28.6	0.96	0.009	0.767	0.010		
BW change,	$-0.71$	$-0.59$	0.198	0.561	0.933	0.001		
kg/day								
Milk composition								
Fat%	4.50	4.49	0.153	0.953	0.414	0.036		
Protein, %	3.49	3.50	0.065	0.933	0.084	${}< 0.001$		
Lactose, %	4.82	4.78	0.032	0.295	0.865	0.006		
Fat, kg/day	1.15	1.23	0.047	0.082	0.882	0.026		
Protein, kg/	0.89	0.96	0.029	0.019	0.796	0.069		
day								
Lactose, kg/	1.24	1.33	0.048	0.088	0.550	< 0.001		
day								
Total solids,	3.47	3.73	0.116	0.029	0.798	0.001		
kg/day								
Total solids, g/	13.6	13.5	0.18	0.988	0.166	0.003		
kg milk								
Urea in milk,	12.6	10.9	0.01	0.077	0.019	0.596		
mg/dL								

Abbreviations: SEM: standard error of means; trt: treatment; DIM: days in milk; DMI: dry matter intake; ECM: energy-corrected milk; BW: body weight.

indicates that appropriate timing of fresh pasture allocation may increase this motivation even further. Interestingly, this slightly differ compared to the findings by Vibart et al. (2017), who only observed a positive impact on grazing when fresh pasture was offered in the morning. In our study, after receiving fresh pasture the PM cows spent 64, 63 and 63 % of their time grazing in the 2, 4 and 6 h windows, respectively. In contrast, the cows in the AM group spent 39, 47 and 40 % of their time grazing in the 2, 4 and 6 h windows respectively. In addition, cows receiving fresh pasture in the afternoon showed a reduction in number of grazing bouts but an increase in the duration of these bouts (Table 3), as also reported by Gregorini et al. (2008,2011), Abrahamse et al. (2009) and Vibart et al. (2017). As a consequence, evening allocation of fresh pasture was significantly more efficient in terms of grazing time in this study.

The ratio of shorter and longer wavelengths when the sun is close to the horizon has been suggested to have a stimulatory effect on appetite (Gregorini et al., 2006). In accordance with this, we observed a shift in activity from ruminating to grazing at sunrise and a major peak in grazing before sunset for both groups. The peaks, seen around 0730 h and 1830 h, could also have been triggered by the milking routine (short-term fasting), as found in other studies (Orr et al., 2001; Iqbal et al., 2023). The high motivation of the cows to graze during the afternoon-early evening, irrespective of fresh pasture allocation time, is in line with findings by Vibart et al. (2017) and Caram et al. (2021). Cows in the AM group spent a similar amount of time grazing during a 4-hour period in morning and afternoon in that study (196 vs 189 min; Vibart et al., 2017) and in our study (109 vs 112 min). When herbivores graze the same area, whether in the wild or in intensive pasture management systems, they often commence grazing collectively at specific times (Molle et al., 2022). However, they generally stop grazing at different times, depending on their different individual needs and/or hunger levels. We observed this pattern (Fig. 3), with low within-group variation in grazing times during the first 2 h after fresh pasture allocation. This variation increased substantially with increasing cumulative time (4 and 6 h), regardless of treatment or milking.

Differences in grazing and rumination behaviours have been associated previously with stage of lactation and parity, due to the different energy requirements in the various physiological states (Iqbal et al., 2023). However, stage of lactation and parity did not influence grazing or rumination duration in our study, although we found an effect of stage of lactation on production parameters and parity is known to impact the time budget of dairy cows (Grant and Albright, 2001). The cows on the study farm have been carefully bred to cope with an intense full-time grazing system and were acclimatised to grazing for more than a month before our sampling, which could possibly explain the lack of parity effect.

Despite the greater total grazing time for cows in the PM group compared with the AM group, estimated intake did not differ significantly between the treatments. However, it is worth noting that herbage intake was estimated using equations based on several assumptions and whether there was a real difference in intake, or not, cannot be exclusively determined by this method.

The total daily rumination time of cows in the PM and AM groups was within the range (387-530 min) reported by Pérez-Prieto and Delagarde (2012). In both groups of cows, most of the rumination took place at night, between dusk and dawn. In addition, there was an increase in rumination activity after each main grazing event, both in the morning and evening (Fig. 2). Interestingly, cows in the PM group showed shorter total daily rumination time than cows in the AM group. This could be a consequence of greater digestibility (Ciavarella et al., 2000; Linnane et al., 2001) and palatability (Provenza et al., 1998), or to more selective grazing behaviour by the PM group, selecting for certain species in the pasture. In line with increased digestibility, Gregorini et al. (2009) reported a decrease in toughness of meadow fescue, and an increase of particle size reduction, from early morning to evening as a consequence of a relative decrease in fibre concentration in the herbage

and an increase in DM and WSC content during the day. Grant et al. (1990) found that a reduction in particle size resulted in shorter rumination time.

Milk yield and daily fat yield were numerically greater in cows in the PM treatment than in AM cows. This numerical increase, in combination with a significant increase in daily protein yield, had a significant effect on ECM, with PM cows producing 10 % more kg ECM than AM cows. Similarly, Vibart et al. (2017) observed a tendency for increased milk fat, milk protein and milk solids yield when the time of allocation to fresh pasture was in the afternoon rather than in the morning.

While a single, simple and easy to adopt change in grazing management appears to be an effective way of increasing milk protein content and ECM yield, results should be considered with caution. Many factors can affect herbage intake and animal performance, such as herbage quality and allowance, sward structure and grazing management, among others. In the present study, the grazing strategy applied by the farmer, similar to a 'rotatinuous' grazing management (Schons et al., 2021), resulted in herbage allowances and pre- and post-grazing sward heights (Table 2) capable of providing the conditions for a maximized herbage intake for both groups of cows.

Assuming that herbage intake was similar for the two groups of cows in our study, the higher yields of ECM and protein may have been due to changes in the chemical composition of the herbage as the day progressed. Better utilisation of dietary N, it is suggested by the tendency (P *<* 0.08) for a lower concentration of urea-N in milk from the PM group than in milk from the AM group. In lactating dairy cows, urea-N in milk can be used as an index of a more optimal balance of energy:protein ratio (Oltner and Wiktorsson, 1983), and of the efficiency of utilisation of dietary N (Gustafsson and Palmquist, 1993; Gonda, Lindberg, 1994; Jonker et al., 1998). Based on the high correlation between MUN and urinary excretion of urea-N (Gonda, Lindberg, 1994; Jonker et al., 1998), a small adjustment in grazing management, would result in a lower environmental impact by reducing excretion of N to the environment (Pozo et al., 2022). However, the fact that in the present study MUN concentrations only tended (p *<* 0.08) to differ between treatments, and dietary-N utilization was not quantified, doesn't allow to draw a clear conclusion on a better efficiency of utilization of N as a result of changing the time of allocation of the daily grazing strip from morning to afternoon as seen by Pozo et al. (2022).

#### **5. Conclusions**

Lactating dairy cows allowed to access their fresh daily grazing strip later in the day devoted more time to grazing and less time to ruminating than cows accessing the fresh pasture early in the morning. This simple change in grazing management from giving access to fresh pasture in the afternoon, rather than the morning, resulted in increased ECM yield, as a result of increased milk protein and total solids yield.

However, before advising the adoption of this simple practice to dairy farmers, more research is needed in order to elucidate how the response in animal performance could be affected by location, seasonality and weather conditions –e.g., photoperiod, heat stress-, pasture characteristics -e.g., botanical composition, phenological stage, herbage mass, sward structure-, and grazing management –e.g., herbage allowance, pre- and post-grazing pasture heights-, among other factors.

#### **CRediT authorship contribution statement**

**E. Ternman:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Q. Lardy:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **R. Danielsson:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **H. Gonda:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

#### **Ethical considerations**

Not applicable. Treatments and handling of animals were part of the everyday farm routine and did not require ethical approval.

#### **Funding**

This project was supported by SLU Ekoforsk, Sweden. Salary for Quentin Lardy was covered by the Norwegian Institute of Bioeconomy Research (NIBIO), Norway.

#### **Declaration of Competing Interest**

Authors declare that there is no conflict of interest

#### **Acknowledgements**

We would like to thank the farmer who let us run this trial on their farm, with all the extra work which that entailed. We are grateful to SLU Ekoforsk for funding the project. We also thank Maria Åkerlind (Växa Sweden) for her valuable assistance in relation to feed calculations, and Elise Neveux (Ecole Supérieure d'Agriculture d'Angers), and Akram Elouardi (Ecole Supérieure d'Ingénieurs en Agroalimentaire de Bretagne atlantique) for help with the botanical composition analyses.

#### **Data availability**

None of the data have been deposited in an official repository, but all are available on request by contacting the corresponding author.

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# Acta Universitatis Agriculturae Sueciae

# DOCTORAL THESIS NO. 2025:05

This thesis evaluates various grazing management systems in Norway and Sweden, focusing on dairy cows' performance, behavior, and methane emissions. It explores a wide range of grazing systems with different proportions of pasture intake in cows' diets during the summer months and investigates how minor adjustments, such as feed timing, can influence behavior and animal performance. The pressing need to reduce emissions from all anthropogenic sources, including the livestock sector, prompted an investigation into the impact of grazed grass compared to grass silage on enteric methane emissions during the grazing season.

**Quentin Lardy** received his postgraduate education in Biological Engineering (option agronomy) at IUT Clermont Auvergne (Aurillac). He received his graduate education at ISTOM Angers, Engineering school of international Agro-Development.

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ISSN 1652-6880 ISBN (print version) 978-91-8046-440-6 ISBN (electronic version) ) 978-91-8046-490-1