



Chicory reduces enteric methane emissions and maintains milk yield but decreases milk fat content compared with perennial ryegrass in dairy cows

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ABSTRACT

The effects of chicory (*Cichorium intybus*) and perennial ryegrass (*Lolium perenne*) diets on methane (CH₄) emissions, feed intake, feeding behavior, and lactation performance in dairy cows were assessed using a randomized complete block design. Thirty-two Holstein-Friesian cows (130 ± 41.9 DIM at the start of the trial; mean ± SD) were assigned to 2 treatments, based on parity, DIM, and fat- and protein-corrected milk (FPCM) yield. The 4-wk experiment included a 2-wk adaptation period followed by 2 wk of data collection. Cows received either early vegetative chicory or late vegetative ryegrass as their sole roughage source, harvested daily and freshly provided in individual feed bins with automated intake recording. Concentrate was provided during milking and via the GreenFeed system that measured gaseous emissions. Compared with ryegrass feeding, chicory feeding did not affect total or roughage DMI, but increased eating time and meal duration, while reducing roughage DM intake rate. Milk fat content decreased by 11% and milk urea content by 52% upon feeding chicory, whereas FPCM, fat, protein, and lactose yields remained unaffected. Methane production (g/d), yield (g/kg DMI), and intensity (g/kg FPCM) were reduced by 26%, 24%, and 25%, respectively. No dietary effect was observed on GreenFeed visit frequency or its influence on CH₄ estimates. Hydrogen production, yield, and intensity was 63%, 68%, and 67% higher, respectively, in cows fed chicory. In conclusion, chicory reduced CH₄ emissions and milk urea content compared with perennial ryegrass, without compromising milk yield, although milk fat content was lowered. Although chicory may serve as a promising forage option to mitigate methane emissions in dairy production systems, the 24% to 26% reduction observed here should be interpreted with caution and

require confirmation across diverse conditions before broader generalization.

Key words: forage, methanogenesis, lactation performance, GreenFeed system

INTRODUCTION

To enhance sustainable productivity and mitigate methane (CH₄) emissions from ruminants, the adoption of herbs in dairy diets has gained increasing attention (Ramírez-Restrepo and Barry, 2005; Hristov et al., 2013). Among these, chicory (*Cichorium intybus*) is considered one of the most promising forage options for grazing livestock due to its nutritional and agronomic benefits (Ramírez-Restrepo and Barry, 2005). As a temperate herb, chicory's taproot structure enables it to access soil moisture in a deeper layer than perennial ryegrass (*Lolium perenne*) during summer drought, helping to partially offset seasonal declines in ryegrass DM yield and supporting milk production in dairy cows (Li and Kemp, 2005; Nwafor et al., 2017). In the past 2 decades, its potential for CH₄ mitigation has attracted increasing interest as an alternative to ryegrass.

Most studies on chicory's effect on CH₄ emissions have been conducted in sheep, often showing reductions in CH₄ yield relative to DMI or digestible DMI (DDMI), rather than CH₄ production (g/d). Waghorn et al. (2002) reported a 37% and 41% lower CH₄ yield (g/kg DMI and g/kg DDMI, respectively) in sheep fed chicory compared with ryegrass–white clover, but these values were derived from separate trials, limiting the validity of direct comparisons due to potential differences in experimental conditions. Niderkorn et al. (2019) reported no change in CH₄ production (g/d), but a 23% reduction in CH₄ yield (g/kg DMI and DDMI) in sheep fed chicory compared with ryegrass. In a study feeding mature chicory and ryegrass to sheep, Sun et al. (2011) observed no significant differences in CH₄ production (g/d) or CH₄ yield (g/kg DMI) between the 2 forages. When using chicory and ryegrass in the vegeta-

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-26. Nonstandard abbreviations are available in the Notes.

tive stage in another study (Sun et al., 2012), no differences in CH₄ production (g/d) or CH₄ yield (g/kg DMI) were observed, but CH₄ yield (g/kg DDMI) was significantly lower (by 8%) with chicory, with a tendency for a more pronounced reduction at 1.3× compared with 2.2× maintenance ME requirement. More recently, Woodmartin et al. (2024a, 2025) found that replacing 25% of ryegrass with chicory had no effect on CH₄ production (g/d) in sheep but significantly reduced CH₄ yield (g/kg DMI) and CH₄ intensity (g/kg ADG). Swainson (2011) reported that sheep fed chicory produced 33% less CH₄ (g/d), 19% less CH₄ (g/kg DMI), and 31% less CH₄ (g/kg DDMI) than sheep fed ryegrass. These findings indicate that the CH₄ mitigation potential of chicory in sheep is influenced by feed intake level and digestibility. In a comprehensive review of dietary methane mitigation strategies, van Gastelen et al. (2019) reported that forage-related mitigation strategies are not equally effective across ruminant species, owing to fundamental differences in intake level and rumen physiology among dairy cattle, beef cattle, and sheep. Therefore, caution is warranted when extrapolating results across ruminant types. In this context, evidence on the effects of feeding chicory on CH₄ emissions from cattle remains limited. To date, only one recent study in dairy-beef heifers has reported that chicory grazing reduced CH₄ production (g/d) and residual CH₄ compared with ryegrass, independent of weaning age (Della Rosa et al., 2026). However, no studies have directly compared CH₄ emissions from chicory and ryegrass in lactating dairy cows.

Animal performance responses to chicory have also been inconsistent. In deer, Kusmartono et al. (1997) reported higher DMI with chicory than with ryegrass, attributing this to chicory's rapid ruminal degradation and rumen outflow rate, which enhance feed intake. On the contrary, Tinworth et al. (1999) suggested that the low DM content of chicory—and resulting bulkiness—may limit DMI in deer. In sheep, Niderkorn et al. (2019) found higher DMI in diets where chicory replaced 50% to 100% of ryegrass, and Woodmartin et al. (2024b) observed an increased DMI when chicory replaced 25% of perennial ryegrass. In dairy cows, no differences in DMI of chicory compared with ryegrass were observed (Muir et al., 2015). Moreover, Minneé et al. (2017) found that replacing 20% or 40% of ryegrass with chicory reduced DMI but sustained similar milk yield in late lactation cows in the first measurement week, whereas by the second week, cows fed chicory had similar DMI and higher milk yield than those fed ryegrass. Despite these inconsistent responses, chicory generally resulted in milk production comparable to ryegrass (Kälber et al., 2011; Muir et al., 2014, 2015; Minneé et al., 2017). However, none of these studies evaluated chicory and ryegrass as the sole roughage source in dairy cow diets.

Based on the above, our objective was to compare the effects of feeding chicory versus ryegrass as the sole roughage source on CH₄ emissions and dairy cow performance, including feed intake, feeding behavior, milk production, and milk composition. We hypothesized that cows fed chicory would emit less CH₄ and consume a similar amount of roughage with no adverse effect on lactation performance.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted from September to October 2022 at the research facility of Wageningen University & Research (Dairy Campus, Leeuwarden, the Netherlands) under the Dutch law on animal experiments in accordance with European Union Directive 2010/63 (EU, 2010). A total of 32 Holstein-Friesian dairy cows were used in a randomized complete block design and paired by parity, DIM, and fat- and protein-corrected milk (FPCM) yield to form 16 blocks, with cows randomly assigned within each block to 1 of 2 dietary treatments: chicory or ryegrass. Of the 32 cows, 10 were in their first lactation, 4 in their second, and 18 in their third or later. The chicory and ryegrass groups had similar baseline characteristics, with average parity 3.4 versus 3.3, DIM 115 versus 118 d, and FPCM yield 37.2 versus 37.2 kg/d, respectively. The experiment was conducted over 4 wk, consisting of a 2-wk adaptation period followed by a 2-wk sampling and measurement period.

Dietary Treatments

During the 2-wk adaptation period, the diet of cows assigned to the chicory treatment was gradually adjusted by replacing ryegrass with chicory, increasing from 33% to 67% of the roughage. During the measurement period, cows were fed either 100% chicory or 100% ryegrass as the sole roughage source. A concentrate (Table 1) formulated to meet the nutritional requirements for maintenance and FPCM yield (37 kg/d on average) was provided, based on net energy for lactation (van Es, 1978) alongside intestinal digestible protein and RDP balance (van Duinkerken et al., 2011; Agrifirm B.V., Apeldoorn, the Netherlands). A total of 1.8 kg/d (DM basis) of concentrate was provided in 2 equal meals at 0700 and 1700 h during milking. Additionally, a bait was supplied during gas emission measurements using the GreenFeed (GF) system (C-Lock Inc., Rapid City, SD). The ingredient and chemical compositions of ryegrass, chicory, concentrate, and GF bait are presented in Table 1. Cows had free access to clean drinking water throughout the trial.

Table 1. Chemical composition (g/kg of DM, unless otherwise stated) of the individual feed ingredients

Item	Ryegrass ¹	Chicory ¹	Concentrate ²	GF bait ³
DM (g/kg of product)	164	81	880	893
OM	886	806	897	933
IVDOM ⁴	663	661	ND ⁴	ND
CP	185	155	161	148
Crude fat	37	32	49	35
NDF	566	314	289	377
ADF	268	245	163	222
ADL	19	24	42	12
Starch	ND	ND	276	96
Sugar	100	120	59	117
Nitrate	0.3	1.7	ND	ND

¹Mineral contents of ryegrass and chicory, respectively: Na (3.2, 6.9 g/kg DM), K (26.7, 53.2 g/kg DM), Mg (2.2, 3.4 g/kg DM), Ca (5.8, 17.2 g/kg DM), P (4.2, 4.3 g/kg DM), and S (4.4, 4.6 g/kg DM); Mn (23.0, 46.5 mg/kg), Zn (30.5, 72.0 mg/kg), Fe (248, 1,089 mg/kg), Cu (10.1, 12.3 mg/kg), Mo (2.0, 1.4 mg/kg), and I (0.6, 1.3 mg/kg); Co (68.5, 393.5 µg/kg DM), and Se (720, 1,510 µg/kg DM).

²Ingredient composition: rapeseed meal = 20 g/kg DM, rumen-protected rapeseed meal = 97 g/kg DM, palm kernel expeller = 310 g/kg DM, CaCO₃ = 37 g/kg DM, mineral salt = 13 g/kg DM, MgO 90% = 6 g/kg DM, NaHCO₃ = 1 g/kg DM, vinasse = 37 g/kg DM, molasses = 44 g/kg DM, wheat middlings = 42 g/kg DM, mineral premix = 2 g/kg DM, wheat = 104 g/kg DM, and corn = 286 g/kg DM.

³GF = GreenFeed. Ingredient composition: sugar beet pulp = 297 g/kg DM, soybean hull = 244 g/kg DM, soyabean meal = 30 g/kg DM, corn gluten meal = 91 g/kg DM, wheat = 75 g/kg DM, alfalfa = 81 g/kg DM, molasses = 38 g/kg DM, barley = 50 g/kg DM, corn = 48 g/kg DM, rapeseed meal = 21 g/kg DM, rumen-protected fat = 14 g/kg DM, and NaCl = 11 g/kg DM.

⁴IVDOM = in vitro digestible organic matter; ND = not determined.

Feeding and Housing

Monoculture chicory pasture was established in May 2022, whereas the 2 ryegrass fields were established in 2010 and 2014 at Wageningen Dairy Campus (Leeuwarden, the Netherlands; 53°10'50.4"N 5°45'23.6"E). Before the feeding trial, chicory and ryegrass were harvested in a staggered manner in August to ensure that forage offered to the cows during the trial (September and October) consisted of 3 to 4 wk of regrowth. During the trial, chicory and ryegrass sward heights were maintained at 25 to 35 cm and 15 to 18 cm, respectively. The soil of the chicory field was a typical sea clay, consisting of 38% clay, 34% silt, and 17% sand, with a pH of 6.6, 78 mg/kg of plant-available K, and 1.2 mg/kg of plant-available P. The soil of the ryegrass fields was a typical sea clay, consisting of 36% clay, 38% silt, and 16% sand, with a pH of 7.1, 105 mg/kg of plant-available K, and 1.4 mg/kg of plant-available P. Ryegrass swards received 230 kg of N/ha of animal manure and 115 kg of N/ha of mineral fertilizer (calcium ammonium nitrate; Rijnvallei, Wageningen, the Netherlands) annually. Chicory sward received 60 kg of N/ha of the same mineral fertilizer annually. During the experimental period, average daily temperature was 14.6°C and monthly total precipitation

was 127 mm. Visual assessment indicated that ryegrass was at a late vegetative stage, with rare seed heads and flowers, whereas chicory was at an early vegetative stage, consisting entirely of leaves with no bolting or flowering stems. Chicory and ryegrass were harvested once daily in the morning by cutting to a height of approximately 6 cm above soil level, weighed, and transported to the feeding alley. The DM content of the harvested ryegrass and chicory was measured daily by drying at 70°C for 36 h. Feed intake for each individual cow was measured using Insentec feed bins (FB; roughage intake control system, Hokofarm Group B.V., Emmeloord, the Netherlands). During the adaptation period, for cows receiving the chicory treatment, chicory and ryegrass were manually mixed in precise ratios before feeding. The FB were manually filled up to 6 times daily with the morning-harvested roughage to ensure a 10% excess over the ad libitum feed intake. The amount of feed offered was calculated based on the feed provided the previous day together with theorts remaining in the FB the following morning (before the first feeding).

Cows were housed as a group in a free stall barn with 32 cubicles, 32 FB for feeding and 3 GF units for CH₄, CO₂, and H₂ measurements. The FB measurement procedure was conducted as described by van Gastelen et al. (2022). In brief, cows were assigned to the FB at the start of the trial and this remained consistent throughout the trial. Each FB was equipped with an automated identification system using transponders in the cows' collars to control access. Experimental diets were equally distributed over the FB across the barn to avoid potential location bias. Cows could access all FB with their assigned diet. For each visit of a cow to a FB, the start and end time of the visit as well as the start and end weight of the FB were recorded. Visits without feed intake were excluded. Weekly, the FB were calibrated by using a standard weight. Feedorts were removed once daily before the first feeding after morning milking. The lying cubicles were fitted with commercially available rubber mats with wood shavings as bedding and were cleaned daily. Cows were exposed to light from 0500 to 2300 h and were milked twice daily at around 0700 and 1700 h in a milking carousel suited for 40 cows with automatic cow identification, milk recording, and automatic cluster removal (AutoRotor PerFormer, GEA Farm Technologies GmbH, Bönen, Germany).

Sample Collection and Measurements

Samples of chicory and ryegrass were taken once daily immediately after cutting, and were stored at −20°C pending analysis. During the evening milking on Tuesday and the morning milking on Wednesday, milk samples (10 mL) were collected into tubes containing sodium azide (5 µL) as preservative, stored at 4°C for

no longer than 1 d, and analyzed for fat, protein, lactose, and urea contents. A weighted average daily milk composition was calculated based on the milk composition from these 2 samples and the corresponding milk yields. The BW and BCS of each cow were recorded twice daily throughout the study using a GEA weighing scale (GEA Farm Technologies GmbH, Bönen, Germany) and a DeLaval BCS camera (DeLaval B.V., Steenwijk, the Netherlands), respectively, both positioned at the exit of the milking carousel with individual identification via a monitor ID system.

Gas Emissions

Emissions of CH₄, CO₂, and H₂ were measured for individual cows using 3 GF units (Zimmerman et al., 2011). Irrespective of treatment, all cows had access to each of these 3 GF units throughout the trial. A sweet, palatable concentrate was offered as bait (Table 1) to encourage the cows to maintain a suitable head position for accurate measurements. The bait was provided from a hopper above the GF hood by using a computer-controlled rotating cup dispenser, with a maximum of 16 so-called cup drops per visit, 1 cup drop per 15 s, and 39.5 ± 2.56 g of concentrate per cup drop. Each individual cow could visit the GF units at intervals of ≥ 3 h (up to 8 visits/d, with the limit reset at 2400 h), and data collection was dependent on the cows' voluntary visits to the GF units. Cows had an average of 56 ± 8.6 (mean $\pm$ SD) valid GF visits during the measurement period, with a minimum of 26 valid GF visits. No cows were excluded from the dataset due to an insufficient number of GF visits (i.e., minimum of 14 measurements; van Gastelen et al., 2024). For each visit, the start and end time of the visit were recorded. Daily GF bait intake per cow was calculated as the number of cup drops consumed multiplied by the average mass per drop. Details regarding GF measurement, calibration, and data management procedures followed the methodology described by Hristov et al. (2015) and van Gastelen et al. (2022). In brief, emissions were calculated by multiplying the difference between background and in-chamber gas concentrations by airflow, and converted to g/d using the ideal gas law with corrections for temperature and pressure. Concentrations of CH₄, CO₂, and H₂ gas were measured using a nondispersive near-infrared analyzer, which was calibrated every third day using a zero-baseline gas of 20% O₂ and 80% N₂ and a span gas mixture of N₂ containing 10 ppm H₂, 1,000 ppm CH₄, 1% CO₂, and 21% O₂ (Linde Gas Benelux B.V., Schiedam, the Netherlands). This calibration was used to remove signal drift in the nondispersive near-infrared analyzer over time by C-Lock Inc., which was found to be negligible. All GF units underwent recovery tests immediately before, midway through, and immediately after the trial,

with each test performed in triplicate for each GF unit. To check the recovery of expired gases, a known amount of CO₂ gas was released into the GF units near the location of the animal's nose during GF visit. The mean CO₂ recovery was 100.2% with individual GF units ranging from 98.0% to 103.9%. Based on these recovery tests, the flow coefficient was adjusted by C-Lock Inc. Statistical analyses were conducted using the validated dataset provided by C-Lock Inc. All GF visit data provided in the validated dataset exceeded 2 min in duration.

Chemical Analysis

At the end of the experiment, chicory and ryegrass samples were thawed at room temperature, pooled by week, thoroughly mixed, subsampled, oven-dried at 70°C until constant weight, and ground to pass a 1-mm screen using a cross beater mill (Peppink 100 AN, Peppink, Olst, the Netherlands). The samples were subsequently analyzed for DM, ash, in vitro digestible organic matter (IVDOM), N, crude fat, NDF, ADF, ADL, sugar, nitrate, and mineral content by Eurofins (Eurofins Agro B.V., Wageningen, the Netherlands). Dry matter content was determined gravimetrically by drying at 104°C for 4 h and ash content by combustion at 550°C for 3 h (International Organization for Standardization [ISO] 6496 [ISO, 1999a] and ISO 5984 [ISO, 2022], respectively). The IVDOM content was determined according to Tilley and Terry (1963). The CP content was calculated as $N \times 6.25$, where N content was determined using the Kjeldahl method with CuSO₄ as catalyst (ISO 5983; ISO, 2005a). Crude fat content was determined by acid hydrolysis followed by extraction with petroleum ether (ISO 6492; ISO, 1999b). The NDF content was determined using the method of van Soest et al. (1991), and ADF and ADL contents were determined according to ISO 13906 (ISO, 2008). Sugar content was determined according to Netherlands Standardization Institute Standard 3571 (Netherlands Standardization Institute, 1974). Nitrate content was determined according to ISO 15923 (ISO, 2013b). Contents of Na, K, Mg, Ca, P, S, Mn, Zn, Fe, Cu, Mo, I, Co, and Se were determined according to ISO 17053 (ISO, 2005b). Milk samples were analyzed for fat, protein, lactose, and urea content by Qlip (Qlip B.V., Zutphen, the Netherlands) using mid-infrared spectroscopy (ISO 9622; ISO, 2013a). The chemical composition of the concentrate and GF bait was calculated based on their ingredient composition and the corresponding nutrient values provided by Centraal Veevoeder Bureau (CVB; CVB, 2023).

Calculations

The ECM yield was calculated as $\text{ECM (kg/d)} = [\text{milk yield (kg/d)} \times 0.327] + [\text{fat yield (kg/d)} \times 12.95] + [\text{pro-}]$

tein yield (kg/d) $\times$ 7.25] (Tyrrell and Reid, 1965). The FPCM yield was calculated as $\text{FPCM (kg/d)} = [0.337 + 0.116 \times \text{fat (g/100 g)} + 0.060 \times \text{protein (g/100 g)}] \times \text{milk yield (kg/d)}$ (CVB, 2022). Daily total DMI was calculated as the sum of roughage, concentrate, and GF bait intake. Feed efficiency was expressed as the ratio of ECM or FPCM yield to total DMI. Gas yield was calculated by dividing daily gas production by total DMI, whereas gas intensity was calculated by dividing daily gas production by ECM or FPCM yield.

After excluding records with negative or zero intake, feeding visit data from the FB and GF units were combined by cow ID, diet group, date, time, and unit, and then sorted by cow ID and visit start time. Visit time was defined as the duration from the start to the end of each visit, and visit interval as the time from the end of one visit to the beginning of the next visit to either a FB or GF unit. Although the GF and FB systems were synchronized to the same barn clock, their recorded time stamps did not align exactly; therefore, a correction of +42 s was applied to the GF data (de Mol et al., 2024). Despite the time correction, 110 negative intervals out of 24,171 visits (0.5%) were still detected. Because such values are physically impossible, these records were excluded from the analysis.

To determine the meal criterion and characterize feeding behavior, intervals between consecutive visits were calculated separately for each cow and natural-log-transformed (Tolkamp and Kyriazakis, 1999). The log-transformed intervals were modeled to 2-population (Gaussian–Gaussian) and 3-population (Gaussian–Gaussian–Weibull [GGW]) distributions using the Finite Mixture Models procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC; Yeates et al., 2001). The GGW model was selected based on visual assessment of fit and a lower -2 log-likelihood, indicating improved model performance. Using the GGW model, the meal criterion (MC; the shortest interval between feeding events that separates 2 consecutive meals) was defined as the intersection point of the second Gaussian and the Weibull component (Abd Rahim et al., 2020). Individual MC values were estimated for each cow, and visits separated by intervals shorter than or equal to the MC were clustered into meals. Intake patterns were calculated on both a daily and a per-meal basis. Eating time was calculated as the sum of visit durations at FB or GF units. Because all cows received the same amount of concentrate in the milking parlor at 0700 and 1700 h, total eating time was defined solely as the total time spent at the FB and GF units throughout the day. Daily roughage DMI and total, FB, and GF visits, as well as total, roughage, and GF bait eating time, were also calculated on a per-meal basis. In addition, for meal-based analysis, meal duration was calculated as the sum of feeding time and nonfeeding interval time within the same meal. Roughage DM intake rate

was calculated as the roughage DMI per meal divided by the corresponding roughage eating time per meal.

Statistical Analysis

Data Exclusion. Parameters related to gas emissions, feed intake and feeding behavior, BW, BCS, and lactation performance were averaged per cow over the 2-wk measurement period. One cow fed chicory was excluded from the trial due to persistent collar recognition problems in both the GF units and the FB system. The CH_4 , CO_2 , and H_2 emission data were screened for outliers using the interquartile range (IQR) method. Values below quartile 1 $- (1.5 \times \text{IQR})$ or above quartile 3 $+ (1.5 \times \text{IQR})$ were classified as outliers, unless a large proportion of such values could be consistently attributed to a specific animal. Ultimately, 73 out of 1,729 GF measurements (4.2%) were identified as outliers and excluded from the dataset.

Data Analysis. Data were analyzed using ANOVA with the MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC). Diet was included as a fixed effect, and block was included as a random effect. All results are presented as $\text{LSM} \pm \text{SEM}$, and significance was declared at $P < 0.05$ and tendencies at $0.05 \leq P \leq 0.10$.

RESULTS

Gas Emissions

Successful voluntary GF visits per cow were fairly evenly distributed over the 24-h period for both the chicory and ryegrass diets, with noticeable decreases particularly during morning milking (Figure 1A). The CH_4 production rate (g/d) was consistently lower in cows fed chicory than in those fed ryegrass throughout the day. Both diets followed a similar pattern, with CH_4 emissions decreasing during the night and gradually increasing after morning milking and feeding (Figure 1B). No association was observed between the number of successful GF visits and measured CH_4 production (Figure 1C).

The CH_4 production (g/d), yield (g/kg DMI), and intensity (g/kg ECM or FPCM) were reduced by 26%, 24%, and 25%, respectively, when feeding chicory compared with ryegrass (Table 2). The H_2 production, yield, and intensity increased by 63%, 68%, and 67%, respectively, in cows fed chicory compared with ryegrass. No differences were observed between diets for CO_2 production (kg/d), yield (g/kg DMI), or intensity (g/kg ECM or FPCM).

Feed Intake and Feeding Behavior

No significant differences were observed for meal criterion, roughage DMI, GF bait DMI, total feed DMI, number of meals, successful visits at FB and GF units

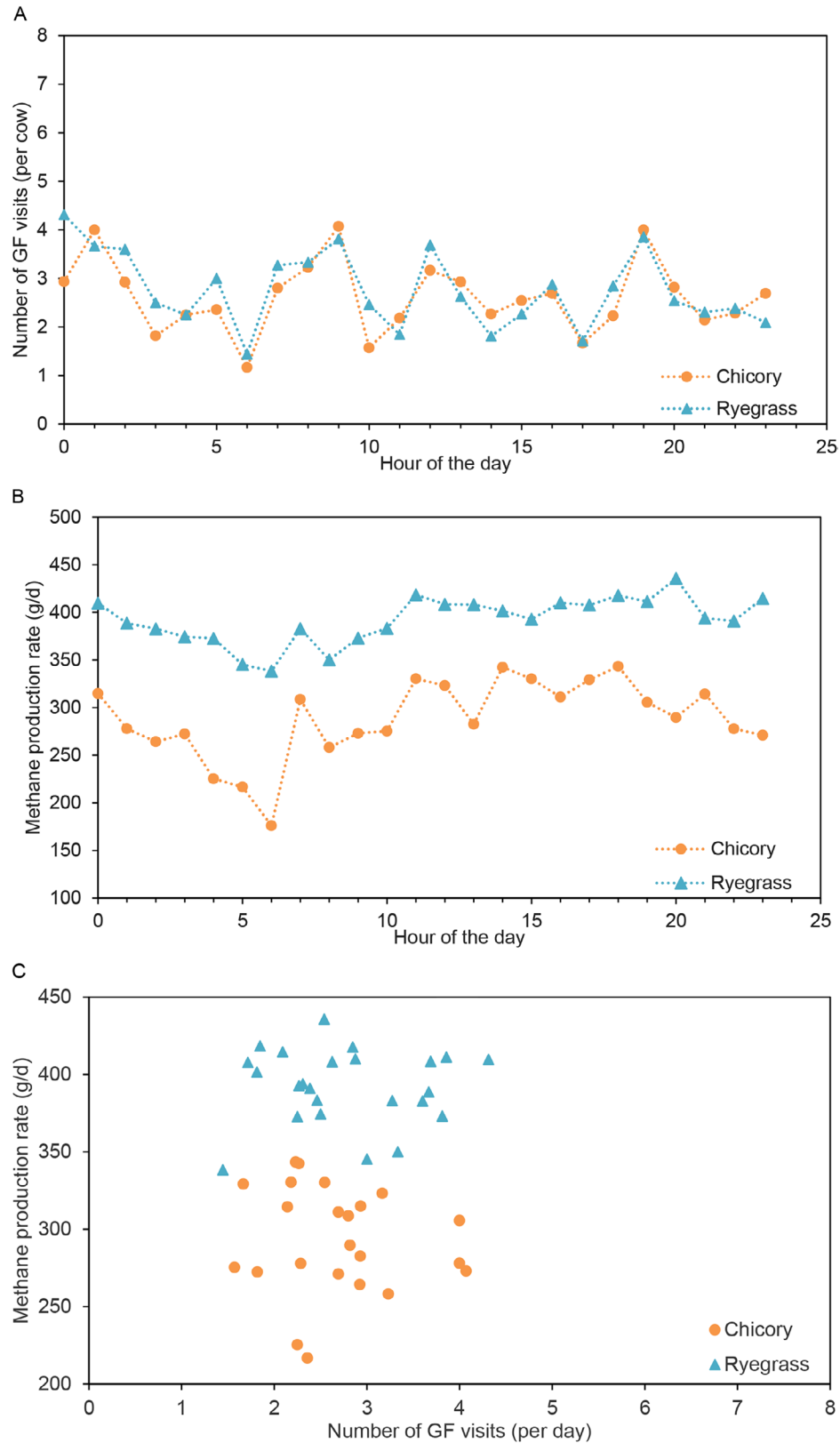


Figure 1. The average number of successful GreenFeed (GF) visits with gas emission measurements per cow by hour of day (A); average CH_4 production rate per cow (g/d) by hour of day (B); and CH_4 production rate per cow (g/d) in relation to daily number of successful GF visits per cow (C) during the 2-wk trial phase.

Table 2. Methane, carbon dioxide, and hydrogen emissions of lactating dairy cows fed ryegrass or chicory diets

	Diet			
Item	Ryegrass	Chicory	SEM	P-value
CO ₂ emission				
Production (kg/d)	12.58	12.59	0.271	0.96
Yield (g/kg of DMI)	630	645	8.7	0.26
Intensity (g/kg of ECM ¹)	395	402	12.1	0.62
Intensity (g/kg of FPCM ²)	430	437	13.2	0.66
CH ₄ emission				
Production (g/d)	393	291	10.9	<0.01
Yield (g/kg of DMI)	19.7	14.9	0.45	<0.01
Intensity (g/kg of ECM)	12.3	9.3	0.34	<0.01
Intensity (g/kg of FPCM)	13.4	10.1	0.37	<0.01
H ₂ emission				
Production (g/d)	0.80	1.30	0.056	<0.01
Yield (g/kg of DMI)	0.040	0.067	0.0024	<0.01
Intensity (g/kg of ECM)	0.025	0.041	0.0018	<0.01
Intensity (g/kg of FPCM)	0.027	0.045	0.0019	<0.01

¹ECM (kg/d) = [milk yield (kg/d) × 0.327] + [fat yield (kg/d) × 12.95] + [protein yield (kg/d) × 7.25] (Tyrrell and Reid, 1965).

²FPCM = fat- and protein-corrected milk. FPCM (kg/d) = [0.337 + 0.116 × fat (g/100 g) + 0.06 × protein (g/100 g)] × milk yield (kg/d; CVB, 2022).

(tendency for visits to GF units), total visits, and feed efficiency (per meal or per day, where applicable; Table 3). Cows fed chicory spent more time eating roughage per meal and per day, which led to greater total eating time and meal duration, and lower roughage intake rate than cows fed ryegrass ($P \leq 0.04$).

BW, BCS, and Lactation Performance

No significant differences in BW, BCS, or milk, ECM, or FPCM yield were observed between cows fed chicory and those fed ryegrass, although milk yield tended to be greater with chicory (Table 4). Milk protein and lactose concentrations did not differ between treatments, but milk fat and urea concentrations were 11% and 52% lower in chicory-fed cows than in ryegrass-fed cows ($P \leq 0.01$).

DISCUSSION

In agreement with our hypothesis, replacing ryegrass entirely with chicory significantly reduced CH₄ production (g/d), yield (g/kg DMI), and intensity (g/kg ECM and FPCM), while maintaining DMI and milk yield, and increasing H₂ emissions. Cows fed chicory showed longer eating times and lower intake rates, indicating behavioral adjustments to cope with the higher bulkiness of the diet. In addition, the lower milk fat content may be related to the reduced fiber concentration and faster rumen fermentation of chicory, which could limit the formation of volatile fatty acids supporting milk fat synthesis.

Gas Emissions

Before analyzing CH₄ emission data, we evaluated whether voluntary visiting behavior at the GF units could have biased the CH₄ measurements, as suggested by de Mol et al. (2024), but found no evidence of such an effect. Diurnal patterns of visit frequency were broadly similar between cows offered chicory and ryegrass, with no consistent visual differences (Figure 1A) and a tendency for a lower number of visits to the GF units for cows offered chicory (Table 3). Additionally, at all hourly intervals (Figure 1B) and across visit frequencies (Figure 1C), cows fed chicory consistently produced less CH₄ compared with those fed ryegrass. To account for potential temporal variation in emissions due to visit timing, CH₄ production was also calculated as a weighted average of each cow's hourly emissions and compared with the simple unweighted mean across all visits per cow. The difference between these 2 approaches was minor, with an average difference of 5.8 g/d, indicating that visit timing had limited effect on CH₄ estimates. The tendency for fewer daily visits to the GF units with chicory compared with ryegrass may be related to the longer meal duration and roughage eating time on chicory, possibly reflecting a lower motivation to obtain the GF bait reward, although causality cannot be inferred from the present experiment as differences in diet composition may also have contributed.

As hypothesized, CH₄ production (g/d) was lower (26%) in cows fed chicory compared with those fed ryegrass. This contrasts with previous findings in sheep (Sun et al., 2011, 2012; Niderkorn et al., 2019) but is consistent with Swainson (2011) and with findings in heifers (Della Rosa et al., 2026). The reduction in CH₄ production may reflect decreased hydrogen production and availability for methanogenesis, which is associated with reduced fiber fermentation in the rumen (Ellis et al., 2008). In our study, chicory had a lower NDF content than ryegrass, consistent with previous findings (e.g., Hoskin et al., 1995; Kusmartono et al., 1997), supporting the interpretation that reduced fiber degradation led to lower hydrogen production and thereby CH₄ production. Additionally, chicory had a higher nitrate content than ryegrass, and nitrate can serve as an alternative hydrogen sink during its reduction to ammonia (van Zijderveld et al., 2011). Based on stoichiometric principles, the greater nitrate concentration in chicory compared with ryegrass (1.7 and 0.3 g/kg DM, respectively) is expected to decrease CH₄ yield by approximately 0.4 g/kg DMI. Collectively, the current findings suggest that lower hydrogen availability contributed most to the lower CH₄ emissions in cows fed chicory.

However, similar to the findings of Della Rosa et al. (2026), cows fed chicory actually emitted more H₂ than

Table 3. Feed intake and feeding behavior of lactating dairy cows fed ryegrass or chicory diets

Item	Diet		SEM	P-value
	Ryegrass	Chicory		
Meal criterion (min)	19.9	17.9	2.83	0.59
Per day				
DMI concentrate ¹ (kg)	1.8	1.8	—	—
DMI roughage (kg)	15.3	14.9	0.51	0.40
DMI GF ² bait (kg)	3.0	2.9	0.12	0.78
DMI total (kg)	20.0	19.6	0.48	0.26
Meals (n)	7.1	7.1	0.32	0.99
Visits total (n)	64.6	76.5	5.78	0.12
Visits FB ² (n)	59.2	71.3	5.71	0.11
Visits GF ³ (n)	5.3	4.8	0.18	0.06
Eating time total (min)	357	429	14.7	<0.01
Eating time FB (min)	331	404	14.5	<0.01
Eating time GF (min)	26	25	1.0	0.31
Feed efficiency (kg of ECM ⁴ /kg of DMI total)	1.61	1.62	0.040	0.69
Feed efficiency (kg of FPCM ⁵ /kg of DMI total)	1.48	1.50	0.037	0.64
Per meal				
DMI roughage (kg)	2.2	2.1	0.14	0.76
Visits total (n)	9.2	10.9	0.83	0.15
Visits FB (n)	8.4	10.2	0.82	0.14
Visits GF (n)	0.77	0.74	0.033	0.44
Eating time total (min)	51	62	3.4	0.03
Eating time FB (min)	47	59	3.3	0.02
Eating time GF (min)	3.7	3.5	0.15	0.25
Meal duration (min)	69	79	4.0	0.04
Roughage DM intake rate (g/min)	46	36	2.2	<0.01

¹Both treatments received the same concentrate during milking, fully consumed by all cows.

²GF = GreenFeed; FB = feed bin.

³Successful GF visits with gas emission measurements.

⁴ECM (kg/d) = [milk yield (kg/d) × 0.327] + [fat yield (kg/d) × 12.95] + [protein yield (kg/d) × 7.25] (Tyrrell and Reid, 1965).

⁵FPCM = fat- and protein-corrected milk. FPCM (kg/d) = [0.337 + 0.116 × fat (g/100 g) + 0.06 × protein (g/100 g)] × milk yield (kg/d; CVB, 2022).

those fed ryegrass. This suggests that, beyond potentially lower hydrogen production and greater hydrogen sinks discussed here, the utilization of hydrogen by methanogens may have been impaired. In addition to the higher nitrate content in chicory, which may lower methanogen abundance (Ma et al., 2025), another possible explanation is the presence of antimethanogenic plant secondary metabolites. In particular, polyphenols and flavonoids are much more concentrated in chicory than in ryegrass (Vasta et al., 2019; Amrit et al., 2023; Rapisarda and Abu-Ghannam, 2023), and have been shown to inhibit methanogen activity (Ramírez-Restrepo and Barry, 2005). Indeed, in several in vitro studies, chicory decreased CH₄ yield per unit DM or digestible DM, often to a greater extent than tannin-rich forages (Loza et al., 2021; Khan et al., 2023).

Beyond nutritional composition, ingestive behavior also influences rumen function and diet utilization in ruminants. Studies in sheep and deer have shown that feeding chicory increases rumen passage rate and reduces retention time compared with ryegrass (Kusmartono et al., 1997; Niderkorn et al., 2019). Shorter retention times of

roughage in the rumen, for example by increasing DMI level, have been associated with decreased CH₄ yield (Warner et al., 2017), faster passage of methanogenic archaea, and increased hydrogen concentrations in the rumen (Janssen, 2010), indicating less complete hydrogen utilization by methanogens. Although these findings in sheep and deer may not directly translate to dairy cows, similar mechanisms may have contributed to reduced methanogenic activity and CH₄ production observed in cows fed chicory in our study.

The substantial reduction (24% to 26%) in CH₄ emissions observed with chicory compared with ryegrass in the present study should be interpreted with caution and not generalized. Forage characteristics, in particular digestibility, are known to affect CH₄ emissions. In dairy cattle, increased digestibility of grass is generally associated with reduced CH₄ yield and intensity (van Gastelen et al., 2019). The ryegrass herbage in the present study was at a late vegetative stage and of relatively low quality, which may have resulted in relatively high CH₄ emissions. When compared with 5-yr average grass herbage data from the Netherlands (2019 to 2023; data

Table 4. Body weight, BCS, and lactation performance of lactating dairy cows fed ryegrass or chicory diets

Item	Diet		SEM	P-value
	Ryegrass	Chicory		
BW (kg)	653	643	17.0	0.49
BCS	2.9	2.9	0.06	0.94
Lactation performance				
Milk yield (kg/d)	27.6	29.4	1.18	0.09
Fat content (g/100 g)	4.56	4.05	0.130	0.01
Protein content (g/100 g)	3.46	3.37	0.059	0.29
Lactose content (g/100 g)	4.46	4.50	0.058	0.69
Urea content (mg/dL)	31	15	1.2	<0.01
Fat yield (g/d)	1,251	1,173	46.4	0.11
Protein yield (g/d)	951	977	30.8	0.27
Lactose yield (g/d)	1,226	1,321	53.5	0.10
ECM ¹ yield (kg/d)	32.1	31.9	1.13	0.80
FPCM ² yield (kg/d)	29.5	29.4	1.04	0.86

¹ECM (kg/d) = [milk yield (kg/d) × 0.327] + [fat yield (kg/d) × 12.95] + [protein yield (kg/d) × 7.25] (Tyrrell and Reid, 1965).

²FPCM = fat- and protein-corrected milk. FPCM (kg/d) = [0.337 + 0.116 × fat (g/100 g) + 0.06 × protein (g/100 g)] × milk yield (kg/d; CVB, 2022).

from Eurofins Agro, Wageningen, the Netherlands), ryegrass used in the present study had a higher NDF content (566 vs. 490 g/kg DM), lower CP (185 vs. 196 g/kg DM) and soluble sugar (100 vs. 130 g/kg DM) contents, and lower in vitro OM digestibility (74.9% vs. 81.1% of dietary OM). Therefore, the CH₄ emissions of the ryegrass in the present study may have been higher than those from an average ryegrass, and consequently the difference between chicory and ryegrass would likely be smaller when compared with higher-quality ryegrass herbage. Chicory also shows considerable variation in nutritive value across maturity stages, seasons, and cultivars, with reported ranges in NDF (346 to 464 g/kg DM; Verma et al., 2022), CP (96 to 145 g/kg DM; Santamaría-Fernández et al., 2019), and IVDOM (532 to 715 g/kg DM; Foster et al., 2002). Compared with these reported ranges, the early vegetative stage chicory used here remains at the higher quality end, with lower NDF (314 g/kg DM) and relatively higher CP (155 g/kg DM) and IVDOM (661 g/kg DM). Consideration of forage variability is therefore essential when evaluating mitigation strategies at the farm, regional or national level (Bannink et al., 2020).

Feed Intake and Feeding Behavior

The hypothesis that DMI would be similar between cows offered chicory and ryegrass diets was confirmed. Consistent with our study, Muir et al. (2015) found no difference in DMI of dairy cows fed either ryegrass or chicory. In contrast, Kusmartono et al. (1997), Swainson (2011), and Niderkorn et al. (2019) reported that feeding 100% chicory improved DMI in red deer and

sheep, respectively. These discrepancies suggest that the effect may differ among animal species, likely due to differences in rumen physiology, digestive capacity, or feeding behavior.

When ryegrass was partly replaced by chicory, Kälber et al. (2011) and Minneé et al. (2017) reported decreased DMI in cows fed chicory. Similarly, Muir et al. (2014) observed a reduced intake with chicory, likely influenced by the supplementation of alfalfa hay before feeding chicory, which could have limited the cows' capacity to consume chicory. Although these studies did not explicitly examine forage preference, it remains a possibility that cows are less inclined to consume chicory when alternative forages are available, even though in the present study we were able to replace all ryegrass with chicory without any limitation to roughage intake. The lower palatability of chicory may be related to the bitter taste of sesquiterpene lactones, which are more abundant in chicory than in ryegrass (Barry, 1998). Consistent with this, dairy cows have been reported to partially avoid chicory, particularly when it was bolting and alternative forages were available (Soder et al., 2006). Cows also showed a lower grazing preference for chicory compared with grasses and legumes in a cafeteria-style experiment (Horadagoda et al., 2009).

In contrast to our study, Mangwe et al. (2019) reported higher DMI when dairy cows were fed 100% chicory compared with a ryegrass-white clover pasture, attributing this to the low bulk density of chicory at the base of the sward, which enabled animals to consume more DM from the available herbage. However, in our study, both chicory and ryegrass were offered under a zero-grazing system, providing equal access to each forage type, which may explain the observed differences. Some other studies have also reported greater DMI with chicory compared with ryegrass; however, these findings often stem from conditions where chicory was included as part of the forage ration, where different animal species were involved, or where animals grazed pastures not composed entirely of ryegrass (Clark et al., 1990; Chapman et al., 2008; McCartney et al., 2014; Murney et al., 2019).

Despite having a similar DMI to cows fed ryegrass, cows fed chicory spent more time eating, had a longer meal duration, and had a lower roughage DMI rate, likely because its lower DM content required the ingestion of a greater bulk of fresh forage to achieve similar DMI. Consistent with this, Della Rosa et al. (2026) reported that heifers grazing chicory spent more time eating than those grazing ryegrass. Gregorini et al. (2013) reported that cows grazing chicory had a lower bite rate and more mastications per bite than cows grazing ryegrass. They suggested that the fresh broad leaves of chicory require more mastication to be accommodated in the oral cavity and formed into a bolus.

Lactation Performance

Consistent with their comparable feed intake, cows fed chicory produced FPCM or ECM yields similar to those fed ryegrass. In the present study, ryegrass and chicory had similar IVDOM contents (663 vs. 661 g/kg DM), which likely contributed to the comparable yields of milk, ECM, and FPCM. This outcome agrees with the findings of Muir et al. (2015), but differ from Mangwe et al. (2019), who reported higher milk yields in cows grazing on chicory compared with ryegrass-white clover pastures. However, their later meta-analysis indicated that the response of milk yield to chicory was largely driven by the control forage type and by DMI (Mangwe et al., 2024), which helps to explain the discrepancy.

Milk fat content was reduced by 11% in cows fed chicory. However, due to a tendency for higher milk yield, milk fat yield remained comparable to that of cows fed ryegrass. The low NDF content and intake of chicory, along with a potentially shorter rumen retention time, may have lowered the intake of physically effective NDF. This, in turn, could have lowered ruminal pH and altered ruminal fatty acid biohydrogenation, thereby promoting the formation of specific intermediates such as *trans*-10 fatty acids, which are known to inhibit mammary lipogenesis (Gaynor et al., 1995; Zebeli et al., 2008; Shingfield et al., 2010). Consistent with this mechanism, Muir et al. (2015) found an increased concentration of *trans*-10 C18:1 in the milk of cows fed chicory compared with ryegrass. In line with this, previous studies also linked similar changes, or the lack thereof, in milk fat content to variations in chicory NDF content (Muir et al., 2014, 2015; Mangwe et al., 2020).

The lower milk urea content observed with chicory compared with ryegrass (15 and 31 mg/dL, respectively) reflects its lower CP content. Elevated plasma urea concentrations may result from excess ruminal ammonia production via microbial deamination of dietary N sources, or from postabsorptive deamination of AA when supply exceeds anabolic demand. Therefore, milk urea levels serve as a useful tool for monitoring N utilization on dairy farms (Nousiainen et al., 2004; Spek et al., 2012). In line with previous studies (Mangwe et al., 2019), we found that chicory contained higher concentrations of dietary minerals, including Na and K, compared with ryegrass (Table 1). Higher Na and K concentrations increase urine volume, thereby diluting urinary N concentration (Bannink et al., 1999; Spek et al., 2012; Letelier et al., 2024). Indeed, several studies have demonstrated that chicory can alter N partitioning in dairy cows and dilute urinary N concentration (Minneé et al., 2017; Mangwe et al., 2019).

Chicory offers advantages for dairy production, including drought resilience due to its deep taproot system (Li

and Kemp, 2005) and reduced CH₄ emissions, as demonstrated in the current study. However, realizing these benefits in practice requires careful attention to grazing management, as chicory presents notable challenges related to relatively poor persistence and sensitivity to grazing management (Badgery et al., 2023). In particular, overgrazing or grazing under wet conditions can damage the crown and taproot, compromising persistency and productivity. Additionally, chicory's low DM content and tendency to bolt in summer can limit forage quality. Under grazing, cows may selectively graze leaves and avoid stems, or intake may be more variable due to sward structure and regrowth stage, potentially modifying the methane mitigation response observed under zero-grazing conditions in the present experiment. Strategic management, including grazing or harvesting chicory at an early vegetative stage, is important to maximize agronomic and environmental benefits (including lower CH₄ emissions) of chicory in dairy production systems.

CONCLUSIONS

Compared with ryegrass, feeding chicory reduced enteric CH₄ emissions and increased H₂ emissions, suggesting a shift in rumen fermentation pathways or inhibition of rumen methanogens by secondary metabolites. Chicory maintained comparable DMI but increased eating time and meal duration, while reducing roughage DM intake rate. Feeding chicory decreased milk urea and fat concentrations without affecting total milk fat and FPCM yield. Overall, these findings indicate that chicory may lower the environmental impact of dairy production systems while maintaining milk component yields; however, the observed responses should be interpreted in the context of the forage stages, with chicory at an early vegetative stage and ryegrass at a late vegetative stage.

NOTES

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Nonstandard abbreviations used: CVB = by Centraal Veevoeder Bureau; DDMI = digestible DMI; FB = feed bin; FPCM = fat- and protein-corrected milk; GF = GreenFeed; GW = Gaussian–Gaussian–Weibull; IQR = interquartile range; ISO = International Organization for Standardization; IVDOM = in vitro digestible OM; MC = meal criterion; ND = not determined.

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