J. Dairy Sci. 109:8435–8442
https://doi.org/10.3168/jds.2025-28158

Effects of lactation stage and behavioral activity on enteric methane emissions in dairy cows

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ABSTRACT

This study investigated how the lactation stage and the main behavioral activities of dairy cows influence enteric methane emissions. The research trial was conducted on 39 Italian Simmental dairy cows reared in a commercial dairy farm. Each animal was monitored during early, mid, and late lactation. Methane emissions were measured using a Laser Methane SMART device (Tokyo Gas Engineering Solutions Ltd.). Simultaneously, eating, rumination, and resting times were continuously recorded using activity monitoring collars. Furthermore, milk yield and composition and feed samples were collected throughout the experiment. Methane emissions were significantly affected by both behavioral activity and its interaction with lactation stage. The highest methane intensity values were observed during rumination in late lactation, whereas resting consistently showed the lowest emissions. Emissions estimated by considering only eating activity tended to overestimate total daily methane output compared with estimates weighted across all behaviors. These findings underscore that behavioral patterns, particularly rumination and resting, contribute substantially to daily methane emissions and should be included in emissions models to improve the accuracy of environmental assessments and mitigation strategies in dairy production systems.

Key words: enteric methane emissions, dairy cow behavior, lactation stage, rumination activity

INTRODUCTION

Increasing concern regarding climate change and environmental sustainability has presented new challenges to the livestock sector, particularly dairy cattle farming. Among the greenhouse gases produced by animals, CH_4 is undoubtedly the most relevant, possessing a global warming potential ~27 times higher than that of CO_2 over a period of 100 yr (IPCC, 2023). Crucially, enteric fermentation is now recognized as the main source of CH_4 of livestock origin, contributing significantly to overall emissions from cattle farms (Gerber et al., 2013; FAO, 2023). Consequently, the reduction of enteric CH_4 has become a primary objective in the pursuit of more sustainable livestock production systems (Beauchemin et al., 2022; Hristov et al., 2022).

A key physiological parameter influencing rumen fermentation is the lactation stage. Multiple studies indicate that the ruminal microbial community is not stable; its composition changes throughout lactation, with direct repercussions on fermentation activity and the amount of enteric CH_4 produced (van Gastelen et al., 2017; Zhu et al., 2021). Generally, emissions are elevated in the early stages of lactation, a period characterized by increased feed intake (Grešáková et al., 2021). However, this stage also coincides with a negative energy balance and a marked mobilization of body reserves, conditions that can reduce the methanogenesis efficiency per unit of ingested feed (Bielak et al., 2016).

In addition to physiological and nutritional aspects, animal behavior can also significantly influence CH_4 emissions (Castaneda et al., 2025). Most existing research has focused on eating time, given its role as a significant moment for CH_4 emission. However, rumination and resting, which together account for a large proportion of a cow's productive day, are often overlooked. During rumination, the rechewing of feed increases saliva production, helping to buffer rumen pH and stabilize fermentation processes, which can in turn affect hydrogen turnover and methanogenesis (Watt et al., 2015; Mikuła et al., 2022). Nonetheless, instruments commonly used to measure emissions, such as sniffer systems, primarily detect eructation associated with feed intake, consequently overlooking the more continuous or subtle release that may occur during rumination or resting. Given that cows spend a relatively limited amount of time eating, whereas rumination and resting take up a much larger portion of the day (Lamanna et al., 2025), focusing only on feeding events may lead to incomplete estimation of total daily CH_4 emissions. Therefore, more research is needed to

Received December 17, 2025.
Accepted April 21, 2026.
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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.

include all behavioral phases in emissions monitoring, to obtain a more complete picture of methane dynamics and improve the accuracy of mitigation strategies.

Building on these premises, the current trial aims to analyze variations in enteric CH_4 production in relation to the different lactation stages and the main behaviors of dairy cows. Furthermore, the potential interaction between lactation stage and behavior will be assessed, to understand how these factors may contribute to the observed differences in overall CH_4 emissions.

MATERIALS AND METHODS

The trial was carried out in a commercial dairy farm in the northeast of Italy. Animals were selected from a herd of 420 dairy cows managed in freestalls and milked 3 times per day in a milking parlor, with free access to feed and water. A total of 39 lactating Italian Simmental cows were used for the experiment. For each animal, all the measurements and the evaluations described herein were performed 3 times: first in the early lactation stage (DIM ≤100), second in mid-lactation (DIM 101 to 200), and finally in late lactation (DIM >200), following the lactation stage duration as per Vijayakumar et al. (2017). At the beginning of each period of measurements BW was estimated following the equation developed by Gruber et al. (2018). Milk production and milk composition were registered during the 3 daily milkings through AfiMilk software (AfiMilk Ltd.).

Animals were herd-reared and fed a TMR mainly composed of corn silage, lucerne hay, and soybean meal, with a chemical composition based on DM: NDF 33.31% DM, ADF 18.49% DM, ADL 7.83% DM, CP 16.03% DM, and ether extract 3.16% DM. Every day of each measurement period, TMR samples were collected immediately after feed distribution, following the procedure described by Robinson and Meyer (2010), and analyzed with a portable near-infrared spectroscopy device, AuroraNir (Grainit s.r.l.). The same diet was offered to the animals for more than a month before the CH_4 measurements. Dry matter intake was estimated following NRC (2001) based on 4% FCM (INRA, 2007), DIM, and BW. Production efficiency was calculated as the ratio of FCM to DMI.

Behavior Parameters and CH_4 Measurements

The animal behaviors evaluated in the study were eating time (**ET**), when the animal was feeding; rumination time (**RT**), when the animal was ruminating; and resting, when the animal was lying on bedding. Each animal involved in the trial wore a collar equipped with specific sensors (SenseHub Dairy, Allflex Livestock Intelligence, Rahway, Merck & Co., USA) that registered the daily behavior data parameters in Lely Horizon v. 4.12.5.609 software (Lely, Maassluis, the Netherlands). The collars registered the minutes of ET and the minutes of RT of each animal during all the period of the trial. The minutes dedicated to non-eating behaviors (other behaviors) were assessed as the difference between total daily minutes and ET and RT. To calculate the daily methane production weighted by the main behaviors, the "resting" methane values were attributed to the minutes of other behaviors.

During the trial, CH_4 measurements were taken twice for each behavior and for each lactation stage, for a total of 18 measurements for each animal. This is a higher number of measurements than generally reported in other studies. For example, Niero et al. (2020), in a study on the repeatability and reproducibility of measurements, performed a total of 15 measurements per subject. The CH_4 was measured with a Laser Methane SMART device (Tokyo Gas Engineering Solutions Ltd.) for 5 consecutive minutes each, as advised by Boré et al. (2022). Figure 1 shows an example of a CH_4 profile collected from the breath of a dairy cow during eating activity. Measurements were taken with the device positioned 1 m from the animal's nostrils, to reduce possible environmental interference and to facilitate the conversion from ppm × m to CH_4 quantity (g/d). The concentrations of CH_4 for each 5-min measurement were calculated using the arithmetic mean of all recorded peaks (Pmean). Estimates of daily CH_4 production were obtained following the equation reported in Sorg et al. (2018):

$$CH_4\ (g/d) = 150.0(\pm 22.10) + 2.2(\pm 0.07) \times \text{Pmean (ppm} \times \text{m)}.$$

Statistical Analyses

Statistical analyses were performed using R software, version 4.1.2 (R Core Team, 2021). The effect of stage of lactation on feeding behavior, milk yield, and milk characteristics was evaluated using a mixed model for repeated measures. The model included stage of lactation (early, mid, late) as a within-subject factor, BW as a covariate, and parity (primiparous, multiparous) as a blocking factor. To assess the effect of stage of lactation and behavior (ET, RT, and others) on methane-related variables, a mixed model for repeated measures was applied. This model included stage of lactation and behavior as within-subject factors, BW as a covariate, and parity as a blocking factor. The interaction between stage of lactation and behavior was also included in the model. Model assumptions for parametric testing were evaluated. Linearity between BW and the dependent variables was assessed by visual inspection of marginal predicted values derived from the mixed model, and the normality of residuals was assessed via Shapiro–Wilk test. Because

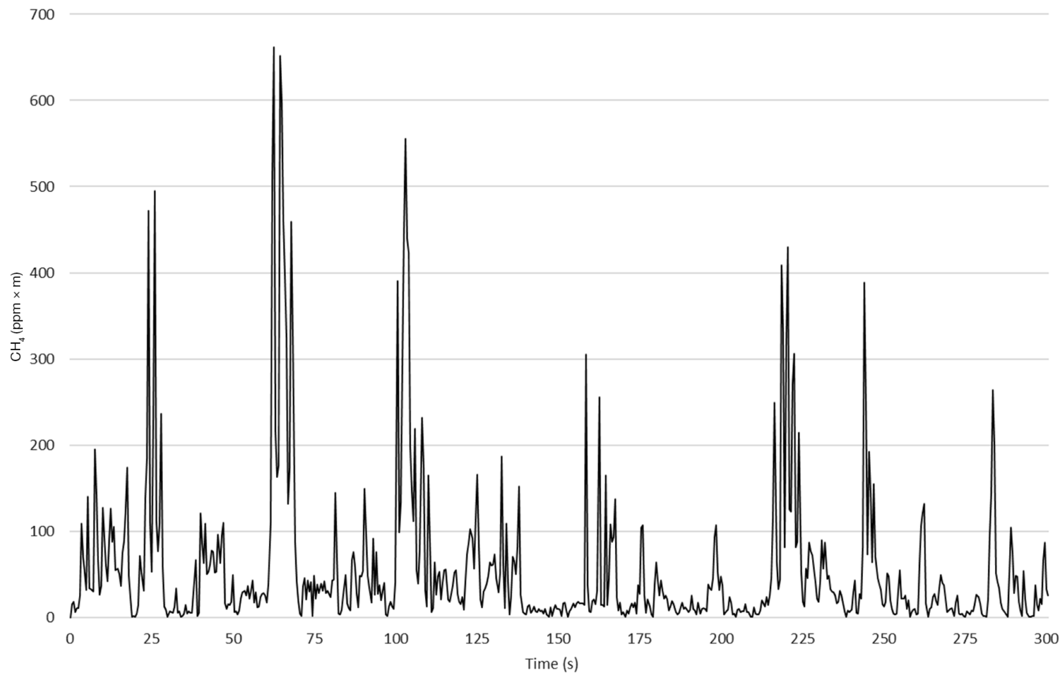

Figure 1. Example of a methane concentration profile measured with Laser Methane SMART (Tokyo Gas Engineering Solutions Ltd.) in the breath of a dairy cow during eating activity.

the normality assumption was satisfied only for milk fat and ET, generalized estimating equations for repeated measures were used for all other variables. Body weight and parity were excluded from the final model if they were not statistically significant. In particular, BW was included as a covariate in the models for milk yield, FCM, DMI, and production efficiency, as well as for comparison between behavior-weighted CH_4 production and that estimated from ET. Parity was included in all models except those for milk yield, FCM, milk fat, ET, and production efficiency. Fisher's LSD test was used as post hoc test, and statistical significance was set at $P < 0.05$.

RESULTS

The results concerning milk yield, milk composition, behavioral parameters and production efficiency are presented in Table 1. Across the 3 lactation stages evaluated in this trial, differences were detected in average daily milk yield, FCM, protein, DMI, and production efficiency ($P < 0.01$). Milk yield and FCM were higher in early and mid-lactation compared with late lactation ($P < 0.01$). Milk protein content resulted in no significant differences between the mid- and late lactation stages (3.52% and 3.58%, respectively) but differed from the protein content in early lactation, which was lower compared with the other stages ($P < 0.01$). No differences were observed in milk fat percentage in milk fat percentage nor in ET and RT durations ($P > 0.05$). The DMI was highest in mid-lactation ($P < 0.01$), but production efficiency decreased from early to late lactation ($P < 0.01$).

Table 2 reports daily CH_4 emissions (g/d), CH_4 yield (g/kg DMI), and CH_4 intensity (g/kg FCM). The CH_4 emissions were affected by cow behavior and its interaction with lactation stage ($P < 0.01$).

The interactions between behaviors and lactation stages are shown in Figure 2, differentiated according to the 3 measurements unit considered: (a) CH_4 emissions (g/d), (b) CH_4 yield (g/kg DMI), and (c) CH_4 intensity (g/kg FCM). Data are shown from the perspective of stage of lactation (left column) and behavior (right column). For CH_4 absolute emissions (Figure 2a), during ET and resting, cows in late lactation exhibited lower CH_4 values compared with those in mid-lactation ($P < 0.05$), whereas cows in early lactation showed intermediate values, not different from the other 2 stages ($P > 0.05$). No differences among stages were detected for RT ($P > 0.05$). When focusing on behaviors, in early and mid-lactation, ET was associated with greater emissions compared with resting ($P < 0.01$), whereas in late lactation, RT had higher values than the other 2 behaviors ($P < 0.01$). For CH_4 yield (g/kg DMI; Figure 2b), differences across lactation stages resulted only for RT, with higher values in late lactation and lower values in mid-lactation ($P < 0.05$), whereas the early stage did not differ from the

Table 1. Estimated marginal means of behavior, milk yield, and milk composition of dairy cows affected by stage of lactation

Item	Stage of lactation			RSD[4]	*P*-value
	Early[1]	Mid[2]	Late[3]		
Milk yield (kg/d)	31.9[B]	30.5[B]	24.0[A]	4.47	<0.01
FCM (kg/d)	31.3[B]	30.5[B]	24.1[A]	4.97	<0.01
Milk composition					
Fat (%)	3.85	3.98	4.03	0.577	0.30
Protein (%)	3.28[A]	3.52[B]	3.58[B]	0.232	<0.01
Behavior					
DMI (kg/d)	22.8[A]	24.0[B]	21.9[A]	1.83	<0.01
ET (min/d)	192.1	184.1	185.6	46.39	0.55
RT (min/d)	523.2	516.1	530.5	52.43	0.46
Production efficiency (FCM/DMI)	1.36[C]	1.27[B]	1.08[A]	0.139	<0.01

[A–C]Within a row, means with different superscripts differ significantly ($P < 0.05$).

[1]DIM ≤100.

[2]DIM 101–200.

[3]DIM >200.

[4]RSD = residual SD; total observations = 117.

other 2. With respect to behaviors, resting consistently displayed the lowest values across all stages ($P > 0.05$), whereas RT had highest values during late lactation ($P < 0.05$). In late lactation, all 3 behaviors differed from each other ($P < 0.05$). Finally, for CH_4 intensity (g/kg FCM; Figure 2c), all behaviors showed higher values in late lactation compared with early lactation ($P < 0.05$). Mid-lactation did not differ from early lactation. Concerning RT, values were higher in late lactation than in early and mid-lactation ($P < 0.01$). Considering emissions across lactation stages, RT did not differ from the other behaviors in early and mid-lactation; however, it predominated in late lactation compared with ET and resting ($P < 0.01$). Both ET and resting presented differences both in early lactation ($P < 0.05$) and in mid-lactation ($P < 0.01$).

Finally, daily methane production estimated based on ET was compared with methane production weighted according to the minutes recorded for the 3 main behaviors. The daily production calculated considering only ET was 449.0 g/d (SE ± 13.1), whereas methane weighted across the 3 behaviors averaged 423.2 g/d (SE ± 11.01). Although the difference was not statistically significant ($P = 0.06$), a tendency was observed for the weighted CH_4 value to be lower than that estimated from feeding time alone.

DISCUSSION

This study investigated the effects of lactation stage and behavior on enteric methane emissions of lactating dairy cows. The study considered early, mid-, and late lactation and the 3 main animal behaviors (eating, ruminating, and resting). The results not only revealed significant differences in milk production and composition during lactation but also highlighted a difference in enteric methane emissions driven by the interaction between the lactation stage and the behavior.

As expected, milk yield peaked in the early lactation stage and progressively declined toward the end of lactation, whereas fat and protein contents generally increased progressively as lactation advanced (Coulon and Rémond, 1991). The current findings are consistent with

Table 2. Estimated marginal means of methane production of dairy cows affected by animal behavior during recording and stage of lactation

Item	Behavior (Beh)			Stage of lactation (SoL)			RSD[4]	*P*-value		
	Eating	Ruminating	Resting	Early[1]	Mid[2]	Late[3]		Beh	SoL	Beh × SoL
CH_4 (g/d)	459.7[B]	468.6[B]	391.6[A]	431.0	464.3	418.5	127.80	<0.01	0.22	<0.01
CH_4 yield (g/kg DMI)	20.4[B]	20.8[B]	17.3[A]	19.4	19.6	19.0	6.05	0.02	0.57	<0.01
CH_4 intensity (g/kg FCM)	17.0[B]	17.3[B]	15.0[A]	14.4[A]	15.6[A]	19.2[B]	6.77	<0.01	<0.01	<0.01

[A,B]Within a row, means with different superscripts differ significantly ($P < 0.05$).

[1]DIM ≤100.

[2]DIM 101–200.

[3]DIM >200.

[4]RSD = residual SD; total observations = 351.

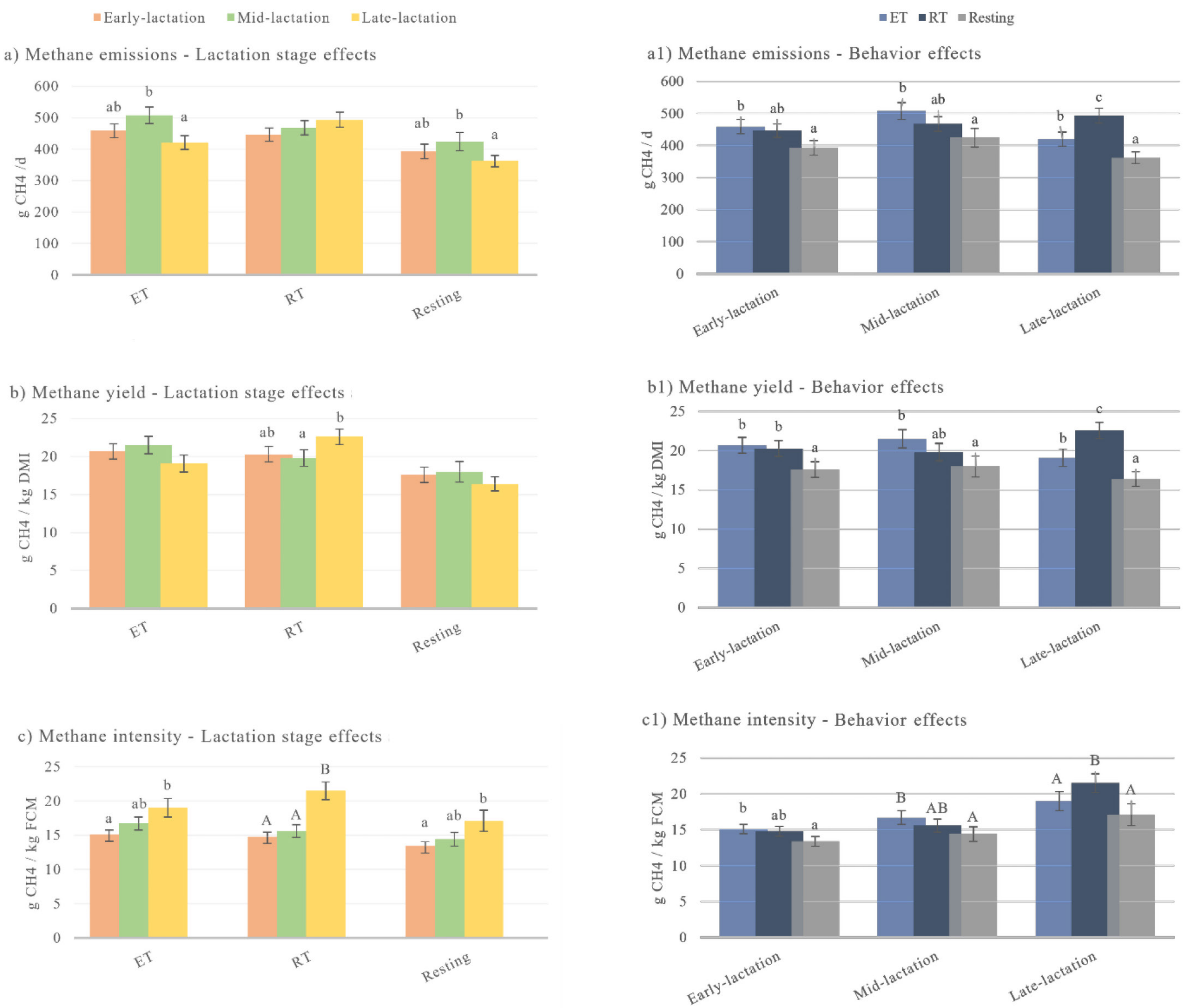

Figure 2. Interactions between behavior and lactation stage for (a) CH_4 emissions (g/d), (b) CH_4 yield (g/kg DMI), and (c) CH_4 intensity (g/kg FCM). The left-hand column shows comparisons among lactation stages within each behavior, whereas the right-hand column shows comparisons among behaviors within each lactation stage. Data are reported as estimated marginal means ± SE. Bars with different letters present a significant difference; bars with shared letters present no significant difference. Uppercase letters (A and B) indicate $P < 0.01$; lowercase letters (a–c) indicate $P < 0.05$.

these established patterns for milk yield and protein content. Conversely, milk fat content did not differ significantly among lactation stages. This partial discrepancy may be attributable to differences in metabolic responses specific to the Simmental breed compared with the Holstein breed, which has been widely used in other studies.

Alongside productive traits, the behavioral variables ET and RT also revealed stage-related patterns. As shown in Table 1, overall ET throughout lactation phases was lower than the value documented by Florit et al. (2023), who established a mean of 227.6 min/d. Average feeding times in the current research did not exceed 192.1 min/d, which was noted in early lactation, with mid- and late-lactation means of 184.1 min/d and 185.6 min/d, respectively. A similar trend was observed for RT. Florit et al. (2023) documented slightly higher average daily rumination times than those in the present trial, where levels ranged from 516.1 to 530.5 min/d ($P = 0.46$) across lactation. Shorter ET and RT may depend on different management practices in the commercial farm (such as milking system or TMR delivery frequency) that influence feeding behavior. Nevertheless, the lack of significant differences in RT is consistent with the findings of Mikuła et al. (2022), who, despite individualizing cows into 3 categories of rumination groups (high, medium, and low), could not find a significant difference in RT during the lactation period. In contrast, a study on Simmental cows dividing lactation into 10 phases reported significant effects of DIM on both ET and RT (Florit et al., 2025). The reduction in production efficiency in late lactation was expected because it was linked to variations in energy partitioning toward milk production and body reserves (Piantoni and VandeHaar, 2023). However, this different efficiency did not affect methane production throughout lactation. Recently, Braidot et al. (2026) also observed that daily CH_4 production is similar among animals with different feed efficiencies.

A useful comparison for the CH_4 yield can be made with the findings of Bittante et al. (2018), who reported reference values of methane emissions across the entire lactation period. However, their estimates were derived indirectly from milk fatty acid profiles, without direct measurements, and did not consider the interaction between behavioral variables and lactation stage. Their study reported an average estimated value of 21.5 g CH_4/kg DMI, which is consistently higher than the measured values obtained in the present trial. Even under the conditions associated with the highest emissions in this study, rumination during late lactation, the average measured value (20.8 g CH_4/kg DMI) remained below the mean reported by Bittante et al. (2018). In contrast, the findings of this study differ from those reported by Grešáková et al. (2021), which suggested an increase in CH_4 production during the early lactation stage, probably due to an increased intake during this stage that was not observed in the present study. Indeed, the DMI was statistically and numerically lower in late lactation than in mid and early lactation, respectively. Conversely, our results showed higher CH_4 intensity values toward the end of lactation. As lactation progresses, feed requirements and feed intake decrease. A lower DMI and a slow ruminal passage rate improve the fermentation efficiency of ingested feed, particularly fiber. It is known that improved fiber fermentation increases the acetate-to-propionate ratio and raises the methane concentration in the fermentation gases (Romanzin et al., 2024; Pavanello et al., 2026).

Dairy cow methane emissions are not constant throughout the day but are influenced by ruminal microbial activity and animal behavior. Chagunda (2013) reported a significant effect of the cow's activity at the time of measurement on enteric methane emissions, with elevated values during behaviors such as drinking and feeding, and lower values during resting and walking. The variation in methane concentration emitted as a function of the cow's behavior is directly linked to the frequency of eructation episodes. During feeding activity, cows are driven to ingest fresh feed (eating) or partially digested feed (rumination), which alters rumen fill and triggers gas release (eructation). The gas contained in the rumen, following ruminal fermentation, has a higher methane concentration than the air exhaled from the lungs during normal breathing cycles (Chagunda, 2013). Consequently, methane emission is lower during rest periods due to reduced eructation frequency.

The interaction between behaviors and lactation stage clearly demonstrates that rumination plays a major role in shaping methane emissions. In terms of absolute emissions (g/d), cows in late lactation displayed the highest CH_4 output during rumination, whereas eating and resting were associated with lower emissions. Similarly, CH_4 yield (g/kg DMI) and methane intensity (g/kg FCM) both reached their maximum in late lactation during rumination, indicating that this behavior becomes progressively more relevant as lactation advances. Conversely, when compared with the findings of Mikuła et al. (2022), a contrasting picture emerges. Their study, based on groups of cows classified by average daily RT, reported that extended rumination was consistently associated with lower methane emissions, both in absolute terms and relative to milk production. In contrast, our results highlight that the relationship between rumination and methane output is strongly dependent on lactation stage. In late lactation, despite prolonged rumination activity, methane values were the highest across all behaviors and measurement units. Further evidence from Bielak et al. (2016) further supports the role of physiological stage. In their study, cows with higher body fat mobilization after 5 wk from parturition showed lower CH_4 yield expressed as CH_4/DMI (29.7 vs. 32.8 L/kg in low-mobilizing cows), a difference that disappeared later in lactation.

The calculation of daily methane production based solely on ET and that weighted according to the 3 main behaviors (ET, RT, and resting) resulted in slightly higher (449 g/d) and lower (423 g/d) values, respectively, compared with the 431 g/d reported by Sorg et al. (2018). Although the weighted methane emissions across the 3 behaviors fall well within the range defined by Sorg et al. (2018), it should be noted that dairy cows in the present study spent an average of 192 min/d eating. Given this limited feeding time, emissions during other behaviors are thought to contribute substantially to the daily total and should be considered when determining overall emissions. Methane measurement systems that sample exhaled air directly at the feeder during feeding time (e.g., GreenFeed, sniffer method) are currently widely used. In contrast, portable infrared instruments, such as the Laser Methane SMART, allow for measurement of CH_4 concentration in exhaled air during other phases as well (Bekele et al., 2022). It should be noted, however, that to convert concentration data to a value comparable to the scientific literature (g/d), an equation based on the GreenFeed comparison (Sorg et al., 2018) is typically applied. Additionally, the trend toward lower weighted CH_4 values suggests that exclusive reliance on feeding-derived estimates may overstate total daily emissions by ~6%. Based on these findings, we consider that it may be useful to consider different cattle behaviors when measuring enteric methane for national or farm inventory purposes. Certainly, further studies are needed to evaluate their global effects and validate appropriate measurement protocols.

NOTES

This study was carried out within the Agritech National Research Center (Naples, Italy) and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022) and with PNRR M4C2, DM352/2022 in collaboration with Le Tenute Marianis farm. The publication was funded by Interdepartmental Research Project CibiAmo (Strategic Plan 2022-25). This manuscript reflects only the authors' views and opinions. Neither the European Union nor the European Commission can be considered responsible for them. The experiment was conducted on one farm in accordance with the European Law (Directive 2010/63/EU) on the protection of animals used for scientific purposes and Italian legislation (DL no. 26, 04/03/2014) and was approved by the ethics committee of the University of Udine (No. 8/2023). The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: ET = eating time; RT = rumination time.

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