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Clustering of feeding strategies to improve the evaluation of enteric and slurry methane emissions in dairy cows: an observational study based on Italian dairy farms

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ABSTRACT

The dairy sector is facing increasing challenges in terms of its environmental impact. Methane (CH_4) is a focal point of research due to its role in enteric emissions from livestock. This study investigates the effects of various feeding strategies on CH_4 emissions from lactating Holstein cows fed total mixed ration (TMR) silage-based diets. Four different equations for estimating CH_4 emissions were chosen according with accuracy and equation variables, and then compared checking whether diet composition has an effect on average emission levels. Only Mills equation detected differences between nutritional clusters. Considering this equation on average, the CH_4 emissions were equal to 460.36 ± 46.95 g/d, 18.90 ± 1.57 g/kg DMI, 12.89 ± 2.83 g/kg FPCM, equal to a loss of 5.93% of gross energy intake. Clustering based on feed composition identified four distinct groups of diets, with no statistically significant difference in CH_4 emissions. The highest emissions were found in the nutritional cluster with higher fibre and starch content, with methane production (MeP) reaching 485.85 g/d, 19.47 kg/kg DMI and 14.82 kg/kg FPCM. This indicates that diet nutrients profile significantly impacts CH_4 emissions, underscoring the importance of adopting sustainable feeding strategies in dairy production. Notably, a positive correlation exists between MeP and milk productivity, while methane intensity negatively correlates with feed efficiency. The findings emphasise the necessity for context-specific emission factors and underscore the importance of implementing sustainable feeding practices to mitigate CH_4 emissions enhancing the efficiency of dairy production systems.

HIGHLIGHTS

- Feeding strategies that enhance feed efficiency and nutrient balance result in reduced methane intensity. Evaluating emissions intensity rather than total production offers a more efficient approach, aligning with sustainability goals while maintaining dairy productivity.
- The type of equation employed to estimate enteric emissions may or may not allow for the identification of the effect of feed strategy on enteric emissions. This underscores the necessity of employing context-specific emission models to more accurately capture dietary impacts on methane production and enhance greenhouse gas inventories.

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Dairy cow; methane; global warming potential; feeding strategy; cluster analysis

Introduction

In recent decades, the dairy sector has faced increasing attention regarding its environmental impact, particularly with respect to greenhouse gas (GHG) emissions. Methane (CH_4) is a significant contributor to global warming, and for this reason it is subject of considerable attention in the definition of sustainable farming practices. However, the newly developed Global Warming Potential star metric (GWP*) indicates that CH_4 has a relatively short atmospheric lifespan and does not lead to significant long-term accumulation (Correddu et al. 2023; del Prado et al. 2023). Although this consideration leads to a reassessment of the impact of CH_4 emissions from livestock, prompting new policy and research directions (Berton et al. 2024), CH_4 remains one of the largest contributors to the global warming potential (GWP) per unit of milk

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produced, followed by emissions associated with feed supply and manure management (Mazzetto et al. 2022).

Currently, there are models available to estimate CH_4 emissions from livestock on both a large scale and at the farm level. However, these tools tend to be very general and lack the flexibility needed to accurately describe specific contexts (IPCC 2019; Colombini et al. 2023). Establishing a CH_4 emissions baseline for a specific production context, such as a particular climate or geographical production system, is valuable for creating national or region-specific inventories, assessing the effectiveness of targeted strategies, and developing predictive models to evaluate potential mitigation measures.

Of further interest is the evaluation of the GWP associated with different feeding strategies, as this offers a comprehensive view of their environmental impact. To date, this has only been investigated by Gislon, Bava, et al. (2020). The identification and implementation of sustainable nutritional strategies are critical for reducing enteric CH_4 emissions while ensuring the long-term sustainability of dairy production systems. Within this context, considerable research has been undertaken with the objective of quantifying enteric emissions to identify the most effective strategies for their reduction. This can be achieved through the use of direct methods (e.g. metabolic chambers, GreenFeed and sniffers) or indirect methods (e.g. *in vitro* studies, predictive models). While direct methods offer high accuracy, they are often impractical for large-scale or commercial applications due to cost and logistical limitations. Indirect methods, including predictive models, provide more feasible alternatives for estimating emissions at both farm and national levels. These indirect approaches rely on phenotypic data, such as diet composition and nutrient/DMI intake, to predict methane production (MeP) (Alemu et al. 2011; Huhtanen et al. 2015; Danielsson et al. 2017; Negussie, de Haas, et al. 2017; Negussie, Lehtinen, et al. 2017; Zhao et al. 2020). However, the accuracy of these models strongly depends on the assumptions made and the specific conditions under which they are developed. The model accuracy variability underscores the need to enhance the predictive power of these models across diverse production systems or to identify the most suitable model for each specific application context (Rossi et al. 2001; Sejian et al. 2011; Hristov et al. 2018). It is therefore of interest to understand whether the effect of a particular diet on emissions may also be influenced by the choice of estimation model (Ellis et al. 2010; Bannink et al. 2011; Sejian et al. 2011; Hristov et al. 2018).

To analyse the relationship between feeding strategies and CH_4 emissions, it is essential to characterise ration composition. Key variables – including the forage-to-concentrate ratio and feed composition – significantly affect the levels of enteric CH_4 emissions (Tavendale et al. 2005; Tamminga et al. 2007; Aguerre et al. 2011; Boadi and Wittenberg 2011; Colombini et al. 2015; Haque 2018; Hristov 2024). Furthermore, animal-specific factors, such as breed, age, physiological stage and health status, also modulate actual CH_4 emissions (Mostert et al. 2018, 2019; Fresco et al. 2023). Additionally, dietary strategies play a crucial role, as they influence nutrient excretion in faeces and urine, which subsequently serve as sources for CH_4 emissions and nitrogen compounds in effluents (Steed and Hashimoto 1994; Wood et al. 2012; Dämmgen et al. 2013; Spek et al. 2013).

The objective of this study was to investigate the association between different feeding strategies, defined by considering either the type of feeds used or nutritional parameters, and CH_4 emissions calculated using the equations proposed in the literature. The study focuses on conventional milk production in Holstein herds fed total mixed ration (TMR) silage-based diets, providing insights for the development of diet-specific emission factors tailored to this production context. In addition, the existing association on the excretion of volatile solids (VSs) and the CH_4 emissions from manure was investigated. To achieve this, four different equations for estimating CH_4 emissions were compared to assess the influence of the chosen estimation tool on the results.

Materials and methods

Dairy farm data collection

The data were collected from 36 dairy farms located in Northern, Central and Southern Italy (30, 1 and 5 farms, respectively). Information on herd composition, agronomic management for self-feeding production (i.e. seeds, diesel, mineral and organic fertilisers, diesel) and purchased feed was collected through a survey for each farm visited once by a trained technician. All data were referred to 2021.

Details of the feed ration used predominantly during the year and labels of the raw materials and concentrates used were collected. These rations were then analysed with Razio-Best (v.560) rationing software (Calamari 2002) to obtain the nutritional parameters, used to apply the enteric CH_4 estimation equations. The nutritional profiles of the diets, as well as their composition in terms of individual feed items, were used to cluster the diets into homogeneous groups. For the composition analysis, individual food items were aggregated into categories: small grain silage (SL; wheat, ryegrass, sorghum, triticale), corn silage (C-SL), hay (HY; wheat, ryegrass, alfalfa, HY mix, meadow, vetch, oat), starch meals (S-ML; corn, wheat, barley triticale, sorghum), protein meals (P-ML; soybean, sunflower, rapeseed), concentrate (CT; compound feeds), byproducts (BYP) and fat (FT).

Data on milk production, FT and protein content were provided by the cooperatives to which the farms delivered their milk. Daily milk production (kg milk) was converted to daily fat and protein corrected milk (FPCM) using the International Dairy Federation (IDF 2015) equation.

Enteric methane emissions

Five equations were selected for predicting daily CH_4 emissions in lactating dairy cows. The choice was based on equations developed in the European context and used in studies to determine the environmental impact of milk production, taking into account the type of variabilities present and their sensitivity to variations in diet (Appuhamy et al. 2016).

The selected prediction equations are: Kirchgessner et al. (KIR) (Kirchgessner et al. 1995), Mills et al. (MIL) (Mills et al. 2003), IPCC Tier 2 2019 (IPCC) (IPCC 2019), IPCC tier 2 2019 with CH_4 conversion rate (MCR) correction (IPCC-MCR) (Dämmgen et al. 2012) and Marumo et al. (MAR) (Marumo et al. 2023). Details are reported in Table 1. To apply the KIR, MIL and MAR equations, the nutritional parameters of the rations provided by the rationing software were used as primary data. In the case of the IPCC-MCR equation, the MCR factor was calculated as a percentage of the actual dietary GE based on CH_4 emissions estimated using the KIR equation (Dämmgen et al. 2012). Enteric CH_4 emissions were then expressed as MeP (g/day), methane intensity (MeI; g/kg FPCM) and methane yield (MeY; g/kg DMI) (Herd et al. 2013).

Volatile solids and methane manure emissions

We calculated VS excretion (kg VS/d) according to the IPCC Tier 2 guidelines (IPCC 2019) using the formula: $VS = [GE \times (1 - DE/100) + (UE \times GE)] \times ((1 - Ash)/18.45)$, where GE represents gross energy intake, DE (%) is diet digestibility, and Ash is the ash content of the diet (IPCC 2019).

Methane emissions for manure storage (kg CH_4/day) were assessed following IPCC Tier 2 approach (IPCC 2019) considering the manure management system in a warm temperature moist climate zone and slurry storage of 3 months and a CH_4 conversion factor (MCR) of 0.24.

Feed and diet GWP calculation

The GWP of the diet was calculated as the sum of the GWP of each feed included in the TMR. The GWP value of each self-produced feed in the sample farms was calculated by means of the LCA (life cycle assessment) method, collecting primary data on agronomic management (seeds, fertilisers, pesticides, diesel). The GWP of purchased feeds, primarily raw materials and concentrates, was sourced from Ecoinvent and AgriFootprint. The inputs were characterised using the GWP100 method with SimaPro software (v.9.5.0), expressing the impact as kg CO_2 eq/kg. The GWP of the diet was reported in units of kg CO_2eq/kg DMI.

Statistical analysis

Data were analysed using R software (R Core Team 2024; version 4.3.3). Before analysis, the normality of distributions was checked with Pearson (Nortest R package). Upon determining non-normality, Spearman's rank correlation test was applied.

To test for the existence of significant differences between the estimates obtained with the five

Table 1. Equations used to predict methane emissions production, expressed as g CH_4/day.

Reference	Methane emission production equations	
Kirchgessner et al. (1995)	$(0.079 \times \text{Fibre (kg/d)} + 0.010 \times \text{NFE (kg/d)} + 0.026 \times \text{CP (kg/d)} - 0.212 \times \text{EE (kg/d)} + 22.995)$	KIR
Mills et al. (2003)	$45.98 - \left(45.98 \times e^{\left(-\left(-0.011 \times \left[\frac{\text{Starch}}{\text{ADF}}\right] + 0.0045\right) \times \text{MEI (MJ/d)}\right)}\right)$	MIL
IPCC Tier 2 (2019)	$\text{GEt (MJ/d)} \times \text{Ym } /55.65$	IPCC
IPCC Tier 2 with MCR (2019)	$\text{GEr (MJ/d)} \times \text{MCR } /55.65$ where $\text{MCR} = -55.65 \times \frac{\text{CH4 (kg/d)}}{\text{GEr (MJ/d)}}$ Kirchgessner et al. (1995)	IPCC-MCR
Marumo et al. (2023)	$34,520 + 16,640 \times DMI \left(\frac{\text{kg}}{\text{d}}\right) + 0.225 \times MFY \left(\frac{\text{g}}{\text{d}}\right) - 0.214 \times MPY \left(\frac{\text{g}}{\text{d}}\right)$	MAR

NFE: nitrogen-free extract; CP: crude protein; EE: ether extract; MEL: metabolic energy for lactation; GEr: real diet gross energy; GEt: theoretical gross energy; MCR: methane conversion factor; MFY: milk fat yield; MPY: milk protein yield; Ym: methane emission factor (IPCC 2019); DMI: dry matter intake.

identified CH_4 prediction equations, the Kruskal–Wallis rank sum tests (package stats) followed by the post hoc Dunn's test with the Bonferroni's correction were applied (FSA R package).

Methane emissions were evaluated considering three milk production classes: 'High' (35 kg FPCM/d/head), 'Medium' (35–30 kg FPCM/d/head) and 'Low' ($\leq$30 kg FPCM/d/head).

A hierarchical cluster analysis was developed to classify diets into homogeneous groups based on their nutritional profile or feed composition. Clustering of the nutritional strategies was based on the type of feed, its level of inclusion (% of dry matter, DM), and its nutritional characteristics (% of dry matter, DM). The number of clusters was determined based on the screen plot and the visual interpretation of the two-dimensional projection of the cluster distribution, in order to identify the configuration that best discriminated among the units. The data were then analysed with Ward method (Stats R package). A p value $\leq$.05 was considered statistically significant for all analyses.

Results

Farm and diet

The selected farms were representative of medium- and large-scale intensive dairy farms in Italy. The farms sample had on average 316 ± 254 (means ± SD) lactating cows and an average annual milk production of 3,895,823 ± 3,168,692 kg of milk with 4.06 ± 0.18% of FT and 3.47 ± 0.08% of protein.

The TMR composition varied in terms of silage and C-SL content, HY, raw materials (starch and protein sources) and CT content according with farm feeding plan. On average, the TMR had DE equal to 73 ± 2% DM, NDF to 35 ± 3%DM, crude protein to 16 ± 2%DM and NE_L of 1.83 ± 0.07 Mcal/kg DM (Table S1).

Three milk classes production were defined in the dataset: 'High' (35 kg FPCM/d/head), 'Medium' (35–30 kg FPCM/d/head) and 'Low' ($\leq$30 kg FPCM/d/head). Some differences but not significant were detected between the three classes in terms of DMI and nutrients content (%DM) of the diet. However, the C-SL inclusion was significantly higher in 'High' group ($p = .020$) where DMI was numerically higher and significantly different feed efficiency (FE) was highlighted compared to the others classes ($p < .001$) (Table 2).

Enteric methane emissions

Enteric CH_4 emissions per head expressed as MeP, MeY and MeI are reported in Table S2. The mean MeP (kg CH_4/d) estimated using IPCC was 456.37 ± 45.10, significantly higher than IPCC-MCR (401.77 ± 54.84) meanwhile MAR showed the highest value (502.14 ± 52.83). Regarding MeY (kg CH_4/kg DMI), estimates obtained using the MAR method indicated the highest values (20.55 ± 0.47), significantly higher than all others methods. The estimate for IPCC and MIL were similar, while IPCC-MCR recorded the lowest value (16.64 ± 3.03) ($p < .001$). The same significant differences were found when CH_4 was expressed as a percentage of the gross energy intake (Ym). When CH_4 was expressed as MeI (kg CH_4/kg FPCM), MAR showed the highest value (13.98 ± 2.49) and IPCC-MCR the lowest (11.19 ± 2.33) ($p < .001$). Overall, the correlation between the estimates obtained with the five equations was tested, revealing a strong correlation for MeI, while the correlations for MeP and MeY were less satisfactory (Table S3).

For IPCC, MIL and MAR most of the considered farms had a MeP ranging from 450 to 500 g/d of CH_4, meanwhile for IPCC-MCR from 400 to 450 g/d of CH_4 and for KIR between 350 and 400 g/d of CH_4. Considering MeI, the higher frequency was between 10 and 13 kg CH_4/kg FPCM for IPCC, IPCC-MCR, MIL and KIR, meanwhile between 13 and 16 kg CH_4/kg FPCM for MAR.

MeI exhibited a negative correlation (ρ) with FE across all model predictions considered (Table S4). In contrast, MeY demonstrated a positive correlation with FE considering IPCC, IPCC-MCR and MAR models (0.86, 0.48 and 0.53, respectively), while it showed a negative correlation applying MIL and KIR models (−0.05 and −0.07, respectively). MeP showed a positive correlation with FE considering IPCC and IPCC-MCR models (0.55 and 0.33, respectively), but a negative correlation with MIL, MAR and KIR models (−0.48, −0.21 and −0.45, respectively).

Considering the milk productivity class, the 'High' production group exhibited a significantly higher MeP than the other groups ($p < .001$) and a greater MeY than the 'Low' production group ($p = .002$) according to the IPCC estimates. For MeI, the lower emissions in the 'High' production group were confirmed by all prediction models, whereas no differences were found between the 'Medium' and 'Low' groups. Additionally, all models except IPCC and IPCC-MCR indicated lower Ym values in the 'High' group than in the 'Low' one. Volatile solid excretion and potential slurry CH_4 production were also higher in the high productivity group ($p \angle .001$) (Table 2).

Ration and nutrients clustering

Four different clusters were identified based on ration composition (Table 3):

Table 2. Enteric methane emissions (means and standard error) expressed as methane production (MeP), methane yield (MeY), methane intensity (MeI) and as percentage of gross energy intake (Ym) according to the identified production classes FPCM (kg/d): high (FPCM $\geq$35 kg/d), medium (FPCM 35–30 kg/d) and low (FPCM $\leq$30 kg/d).

	Item	Unit	High	Medium	Low	*p* Value
	Farm	n°	24	6	6	
	Milk	kg FPCM/d	40.30 (0.51)^{b}	32.62 (0.53)^{a}	26.84 (1.43)^{a}	<.001
MeP	IPCC	g/d	480.86 (6.21)^{b}	423.64 (6.45)^{a}	391.15 (8.78)^{a}	<.001
	IPCC-MCR	g/d	415.73 (10.73)	382.76 (24.50)	364.95 (17.02)	.096
	MIL	g/d	461.86 (10.05)	455.49 (19.58)	459.25 (18.01)	.995
	MAR	g/d	511.02 (11.51)	493.97 (23.06)	474.79 (7.74)	.139
	KIR	g/d	421.47 (11.92)	428.27 (19.56)	438.61 (17.64)	.732
MeY	IPCC	g/DMI	19.61 (0.43)^{b}	17.65 (0.67)^{ab}	16.76 (0.50)^{a}	.002
	IPCC-MCR	g/DMI	17.03 (0.64)	16.07 (1.39)	15.66 (0.89)	.485
	MIL	g/DMI	18.71 (0.27)	18.88 (0.67)	19.70 (0.96)	.152
	MAR	g/DMI	20.65 (0.10)	20.41 (0.17)	20.30 (0.13)	.159
	KIR	g/DMI	17.06 (0.37)	17.82 (0.90)	18.79 (0.89)	.198
MeI	IPCC	g/kg FPCM	11.95 (0.14)a	13.00 (0.29)^{b}	14.80 (0.93)^{b}	<.001
	IPCC-MCR	g/kg FPCM	10.35 (0.28)a	11.79 (0.87)^{ab}	13.94 (1.41)b	.007
	MIL	g/kg FPCM	11.49 (0.27)a	13.99 (0.66)^{b}	17.41 (1.29)b	<.001
	MAR	g/kg FPCM	12.70 (0.28)a	15.13 (0.55)^{b}	17.92 (0.89)b	<.001
	KIR	g/kg FPCM	10.49 (0.30)a	13.16 (0.70)^{b}	16.74 (1.59)b	<.001
Ym	Ym-IPCC	% GEI	5.80 (0.04)	5.85 (0.07)	5.95 (0.09)	.166
	Ym-IPCC-MCR	% GEI	5.01 (0.12)	5.27 (0.29)	5.56 (0.29)	.189
	Ym-MIL	% GEI	5.57 (0.11)^{b}	6.30 (0.29)^{ab}	6.98 (0.25)^{a}	.001
	Ym-MAR	% GEI	6.17 (0.13)^{b}	6.82 (0.32)^{ab}	7.23 (0.12)^{a}	.002
	Ym-KIR	% GEI	5.08 (0.13)^{b}	5.90 (0.20)^{ab}	6.69 (0.32)^{a}	<.001
	VS IPCC	kg/d	7.25 (0.13)^{b}	6.39 (0.27)^{a}	6.02 (0.23)^{a}	<.001
	CH_4 slurry	kg/d	0.34 (0.01)^{b}	0.30 (0.01)^{a}	0.28 (0.01)^{a}	<.001
	Diet GWP	kg CO_{2eq}/kg DMI	0.76 (0.02)	0.76 (0.10)	0.66 (0.06)	.477
Nutrients	DMI	kg/d	25.00 (0.65)	24.23 (1.13)	23.39 (0.46)	.269
	DE	%DM	73.46 (0.37)	73.17 (0.95)	71.83 (1.14)	.421
	OMI	%DM	94.85 (0.12)	94.68 (0.14)	94.46 (0.25)	.412
	dOM	%OMI	76.02 (0.27)	75.91 (0.80)	74.24 (1.18)	.329
	Crude Fibre	%DM	15.77 (0.25)	15.84 (0.71)	17.69 (0.98)	.113
	NDF	%DM	34.91 (0.41)	34.91 (1.38)	36.52 (1.66)	.364
	ADF	%DM	19.17 (0.30)	19.70 (0.87)	21.29 (1.24)	.113
	CP	%DM	15.90 (0.39)	15.96 (0.76)	14.94 (0.84)	.695
	Starch	%DM	29.93 (0.58)	30.01 (1.44)	27.77 (1.99)	.186
	EE	%DM	3.79 (0.15)	3.49 (0.29)	3.57 (0.28)	.611
	NFE	%DM	59.25 (0.44)	59.15 (0.98)	58.04 (0.90)	.443
	Ash	%DM	5.15 (0.12)	5.32 (0.14)	5.54 (0.25)	.412
	F/C	%DM	38.48 (0.92)	42.73 (3.38)	46.23 (4.51)	.215
	NE_L	Mcal/kg DM	1.84 (0.01)	1.83 (0.04)	1.78 (0.04)	.388
	FE	kg FPCM/kg DM	1.65 (0.04)^{b}	1.36 (0.05)^{a}	1.15 (0.05)^{a}	<.001
	MEI	MJ/d	297.87 (6.75)	289.79 (16.61)	273.86 (9.35)	.336
Feed	SL	%DM	51.84 (1.83)	42.56 (5.95)	43.40 (3.47)	.091
	C-SL	%DM	40.43 (1.83)^{b}	33.86 (3.52)^{ab}	30.01 (2.66)^{a}	.020
	HY	%DM	9.84 (1.31)	15.64 (5.54)	17.80 (5.54)	.282
	S-ML	%DM	10.10 (1.70)	16.06 (3.54)	18.67 (4.44)	.110
	P-ML	%DM	8.99 (1.62)	7.22 (1.81)	10.66 (4.24)	.760
	CT	%DM	17.84 (3.00)	18.33 (5.16)	7.30 (3.37)	.233
	BYP	%DM	0.61 (0.32)	0.00 (0.00)	1.96 (1.36)	.295
	FT	%DM	0.70 (0.26)	0.20 (0.20)	0.21 (0.21)	.466
	AD	%DM	0.07 (0.05)	0.00 (0.00)	0.00 (0.00)	.337

FPCM: fat protein corrected milk; MeP: methane production; MeY: methane yield; MeI: methane intensity; Ym: methane conversion factor; IPCC: enteric methane IPCC (Tier 2, 2019); IPCC-MCR: enteric methane IPCC (Tier 2, 2019) with methane conversion ratio from Kirchgessner et al. (1995); MIL: enteric methane from Mills et al. (2003); MAR: enteric methane from Marumo et al. (2023); KIR: enteric methane from Kirchgessner et al. (1995); VS: volatile solids; GWP: global warming potential; CO_{2eq}: carbon dioxide equivalent; DE: digestible energy; OMI: organic matter intake; dOM: digestible organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; ^{19}CP: crude protein; EE: ether extract; NFE: nitrogen-free extract; F/C: forage concentrate ratio; NE_L: net energy for lactation; FE: feed efficiency; MEI: metabolisable energy intake; SL: total silage; C-SL: corn silage; HY: hay; S-ML: starch meals; P-ML: protein meals; CT: concentrate; BYP: by-products; FT: fat; AD: additives.

Means within a row with different superscripts (a, b) differ (*p* value $\leq$.05).

- *Cluster 1 (CL-F1)*: Characterised by a higher inclusion of HY (20 ± 3%DM, $p = .007$), S-ML (22 ± 1.8%DM, $p < .001$) and P-ML (13 ± 1.8%DM, $p < .001$), along with a lower content of SL (37 ± 1.7%DM, $p < .001$), C-SL (31 ± 1.5%DM, $p = .004$) and CT (7 ± 3%DM, $p < .001$).
- *Cluster 2 (CL-F2)*: Noted for medium levels of SL (50 ± 2.6%DM, $p < .001$), C-SL (38 ± 3.1%DM, $p = .004$) and HY (8 ± 2.9%DM, $p = .007$), combined with a higher CT content (37 ± 2.1%DM, $p < .001$).
- *Cluster 3 (CL-F3)*: Characterised by a higher level of SL (54 ± 2.4%DM, $p < .001$), medium levels of C-SL

Table 3. Average feed content and nutritional value of the diet in the feed clusters (CL-F).

			Feed cluster				
	Item	Unit	CL-F1	CL-F2	CL-F3	CL-F4	p Value
	Farm	n°	12	7	8	9	
	FPCM	kg/d	33.84 (1.85)	39.10 (1.39)	36.72 (2.23)	38.93 (1.73)	.204
Feed	SL	%DM	37.41 (1.67)^{a}	50.25 (2.57)^{ab}	53.71 (2.37)^{b}	58.84 (1.98)^{b}	<.001
	C-SL	%DM	30.78 (1.54)^{a}	38.10 (3.13)^{ab}	37.31 (2.91)^{ab}	46.54 (2.83)^{b}	.004
	HY	%DM	19.66 (2.97)^{b}	7.78 (2.90)^{ab}	10.39 (2.42)^{ab}	7.02 (1.95)^{a}	.007
	S-ML	%DM	21.88 (1.81)^{b}	1.16 (0.83)^{a}	13.10 (1.50)^{bc}	8.37 (2.02)^{ac}	<.001
	P-ML	%DM	13.16 (1.84)^{b}	2.50 (1.74)^{a}	1.17 (1.17)^{a}	15.37 (1.25)^{b}	<.001
	CT	%DM	7.44 (3.00)^{a}	37.00 (2.10)^{b}	20.33 (1.97)^{ab}	7.90 (2.31)^{a}	<.001
	BYP	%DM	0.00 (0.00)	0.79 (0.66)	1.15 (0.77)	1.30 (0.94)	.321
	FT	%DM	0.45 (0.32)	0.49 (0.23)	0.16 (0.16)	1.03 (0.53)	.438
	AD	%DM	0.01 (0.01)	0.03 (0.03)	0.00 (0.00)	0.16 (0.14)	.492
Nutrients	DMI	kg/d	24.02 (0.91)	25.51 (0.99)	24.54 (0.62)	24.74 (1.24)	.624
	DE	%DM	72.33 (0.70)	73.29 (0.61)	72.88 (0.61)	74.33 (0.67)	.208
	OMI	%DM	94.73 (0.14)	94.54 (0.18)	94.60 (0.21)	95.11 (0.22)	.223
	dOM	%OMI	75.65 (0.66)	75.73 (0.60)	75.12 (0.69)	76.27 (0.38)	.684
	Crude fibre	%DM	16.43 (0.59)	15.57 (0.52)	16.48 (0.63)	15.75 (0.41)	.767
	NDF	%DM	34.79 (0.91)	35.53 (0.90)	36.21 (1.09)	34.52 (0.56)	.757
	ADF	%DM	20.10 (0.68)	19.06 (0.75)	20.04 (0.70)	19.02 (0.51)	.537
	CP	%DM	16.23 (0.57)	15.53 (0.89)	14.88 (0.63)	16.05 (0.51)	.549
	Starch	%DM	29.13 (1.13)	30.02 (1.23)	29.69 (1.09)	29.75 (1.09)	.908
	EE	%DM	3.53 (0.20)	3.85 (0.19)	3.43 (0.15)	4.07 (0.31)	.278
	NFE	%DM	58.30 (0.59)	59.49 (1.08)	59.61 (0.56)	59.13 (0.77)	.463
	Ash	%DM	5.27 (0.14)	5.46 (0.18)	5.40 (0.21)	4.89 (0.22)	.223
	F/C	%DM	44.15 (2.48)^{b}	37.30 (1.88)^{ab}	41.90 (2.28)^{ab}	36.79 (1.32)^{a}	.033
	NE_L Mcal/kg DM		1.81 (0.03)	1.84 (0.02)	1.81 (0.02)	1.87 (0.02)	.194
	FE kg FPCM/kg DM		1.43 (0.10)	1.55 (0.05)	1.50 (0.08)	1.61 (0.10)	.388
	MEI	MJ/d	283.44 (11.30)	302.43 (8.53)	290.41 (7.43)	298.81 (13.83)	.588

FPCM: fat protein corrected milk; SL: total silage; C-SL: corn silage; HY: hay; S-ML: starch meals; P-ML: protein meals; CT: concentrate; BYP: by-products; FT: fat; AD: additives; DE: digestible energy; OMI: organic matter intake; dOM: digestible organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; CP: crude protein; EE: ether extract; NFE: nitrogen-free extract; F/C: forage concentrate ratio; NE_L: net energy for lactation; FE: feed efficiency; MEI: metabolisable energy intake.
Means within a row with different superscripts (a, b) differ (p value $\leq$.05).

(37 ± 2.9%DM, $p = .004$), HY (10 ± 2.4%DM, $p = .007$), S-ML (13 ± 1.5%DM, $p < .001$) and CT (20 ± 2%DM, $p < .001$), and a lower content of P-ML (1 ± 1.2%DM, $p < .001$).

- *Cluster 4 (CL-F4)*: Defined by higher inclusions of SL (59 ± 2%DM, $p < .001$), C-SL (47 ± 2.8%DM, $p = .004$) and P-ML (15 ± 1.3%DM, $p < .001$), with lower levels of HY (7 ± 1.9%DM, $p = .007$), S-ML (8 ± 2%DM, $p < .001$) and CT (8 ± 2.3%DM, $p < .001$).

The F:C ratio was significantly different in the four clusters, with CL-F1 showing the highest ratio and CL-F4 the lowest ($p = .033$). Despite variations in milk production and FE, no statistically significant differences were found. Additionally, no significant differences were observed in MeP, MeY, MeI and Ym although clusters with higher production had the lowest MeY, and those with higher SL and C-SL inclusion exhibited the lowest MeP and MeY (Table 4).

Three different clusters were identified based on nutrient composition (Table 5):

- *Cluster 1 (CL-N1)*: Characterised by medium DE (73 ± 0.4%DM, $p < .001$) and NDF (35 ± 0.5%DM, $p < .001$) levels, higher content of fibre (16 ± 0.3%DM, $p < .001$), ADF (20 ± 0.4%DM, $p < .001$) and CP (17 ± 0.3%DM, $p < .001$) and NEL (1.84 ± 0.2 Mcal/kg DM, $p < .001$), and a lower starch content (27 ± 0.7%DM, $p < .001$).
- *Cluster 2 (CL-N2)*: Exhibits the highest digestibility (DE and dOM, 75 ± 0.3%DM and 77 ± 0.3%OMI, respectively) and NEL (1.87 ± 0.01 Mcal/kg DM, $p < .001$), with the lowest levels of fibre (15 ± 0.3%DM, $p < .001$), NDF (33 ± 0.5%DM, $p < .001$) and ADF (18 ± 0.4%DM, $p < .001$), and higher starch content (33 ± 0.5%DM, $p < .001$).
- *Cluster 3 (CL-N3)*: Shows the lowest DE (71 ± 0.5%DM, $p < .001$), higher OMI (95 ± 0.2%DM, $p < .001$) and fibre (18 ± 0.5%DM, $p < .001$), NDF (38 ± 0.6%DM, $p < .001$) and ADF (21 ± 0.5%DM, $p < .001$) and NEL (1.75 ± 0.02 Mcal/kg DM, $p < .001$). But lower dOM (74 ± 0.5%OMI, $p < .001$), along with lower levels of CP (14 ± 0.4%DM, $p < .001$) and starch (27 ± 0.5%DM, $p < .001$).

Considering the ration characteristics of these cluster, the HY content varied resulting medium in the CL-N1 (11 ± 2.5%DM), lower in the CL-N2 (7 ± 1.02%DM) and higher in CL-N3 (21 ± 3.56%DM) ($p = .003$). Despite variations in milk production and FE, no statistically significant differences were found. Methane

Table 4. Enteric methane emissions (means and standard error) expressed as methane production (MeP), methane yield (MeY), methane intensity (MeI) and as percentage of gross energy intake (Ym), volatile solids (VS) excretion, methane emissions from slurry storage and diet GWP in the feed clusters (CL-F).

			Cluster				
	Item	Unit	CL-F1	CL-F2	CL-F3	CL-F4	*p* Value
MeP	IPCC	g/d	439.73 (13.40)	485.49 (16.78)	456.63 (16.89)	455.70 (12.02)	.157
	IPCC-MCR	g/d	404.07 (16.26)	400.52 (17.49)	420.59 (16.70)	382.96 (22.30)	.485
	MIL	g/d	455.06 (13.86)	463.40 (20.78)	464.07 (11.84)	461.78 (18.74)	.949
	MAR	g/d	493.56 (18.27)	517.22 (15.78)	501.90 (13.67)	502.08 (20.49)	.725
	KIR	g/d	433.06 (14.38)	420.50 (21.04)	444.10 (15.69)	402.63 (21.31)	.457
MeY	IPCC	g/DMI	18.59 (0.79)	19.29 (0.56)	18.64 (0.50)	18.88 (0.91)	.786
	IPCC-MCR	g/DMI	17.24 (1.09)	15.89 (0.52)	17.19 (0.61)	15.94 (1.27)	.477
	MIL	g/DMI	19.15 (0.58)	18.37 (0.61)	18.97 (0.37)	18.93 (0.48)	.666
	MAR	g/DMI	20.63 (0.13)	20.52 (0.17)	20.48 (0.12)	20.55 (0.21)	.913
	KIR	g/DMI	18.23 (0.60)	16.63 (0.38)	18.15 (0.55)	16.52 (0.80)	.102
MeI	IPCC	g/kg FPCM	13.29 (0.59)	12.44 (0.28)	12.60 (0.45)	11.81 (0.31)	.084
	IPCC-MCR	g/kg FPCM	12.30 (0.83)	10.24 (0.26)	11.68 (0.68)	9.99 (0.69)	.058
	MIL	g/kg FPCM	13.93 (0.95)	11.98 (0.81)	13.02 (0.99)	12.12 (0.87)	.240
	MAR	g/kg FPCM	15.05 (0.92)	13.28 (0.41)	13.95 (0.76)	13.10 (0.75)	.274
	KIR	g/kg FPCM	13.39 (1.13)	10.79 (0.49)	12.47 (1.03)	10.58 (0.81)	.180
Ym	Ym-IPCC	% GEI	5.85 (0.06)	5.96 (0.10)	5.81 (0.05)	5.73 (0.03)	.203
	Ym-IPCC-MCR	% GEI	5.38 (0.19)	4.91 (0.12)	5.37 (0.17)	4.82 (0.25)	.083
	Ym-MIL	% GEI	6.09 (0.20)	5.72 (0.34)	5.97 (0.30)	5.85 (0.30)	.703
	Ym-MAR	% GEI	6.61 (0.27)	6.36 (0.17)	6.42 (0.19)	6.34 (0.28)	.869
	Ym-KIR	% GEI	5.82 (0.27)	5.17 (0.22)	5.71 (0.31)	5.09 (0.29)	.303
	VS IPCC	kg/d	6.80 (0.25)	7.15 (0.32)	6.99 (0.32)	6.75 (0.19)	.566
	CH_4slurry	kg/d	0.32 (0.01)	0.33 (0.01)	0.33 (0.01)	0.31 (0.01)	.566
	Diet GWP kgCO_{2eq}/kg DMI		0.73 (0.06)	0.83 (0.04)	0.73 (0.03)	0.72 (0.04)	.232

MeP: methane production; MeY: methane yield; MeI: methane intensity; Ym: methane conversion factor; IPCC: enteric methane IPCC (Tier 2, 2019); IPCC-MCR: enteric methane IPCC (Tier 2, 2019) with methane conversion ratio from Kirchgessner et al. (1995); MIL: enteric methane from Mills et al.(2003); MAR: enteric methane from Marumo et al.(2023); KIR: enteric methane from Kirchgessner et al. (1995); FPCM: fat protein corrected milk; GEI: gross energy intake; VS: volatile solids; GWP: global warming potential; CO_{2eq}: carbon dioxide equivalent.
Means within a row with different superscripts (a, b) differ (*p* value $\leq$.05).

Table 5. Average feed content and nutritional value of the diet in the nutrient clusters (CL-N).

			Cluster			
	Item	Unit	CL-N1	CL-N2	CL-N3	*p* Value
	Farm	n°	10	16	10	
	FPCM	kg/d	36.37 (1.81)	38.58 (1.14)	34.29 (2.33)	.294
Nutrients	DMI	kg/d	24.03 (0.43)	24.73 (0.93)	24.97 (0.83)	.852
	DE	%DM	72.9 (0.43)^{ab}	74.69 (0.30)^{b}	70.9 (0.55)^{a}	<.001
	OMI	%DM	94.4 (0.14)^{a}	95.14 (0.11)^{b}	94.51 (0.18)^{a}	.001
	dOM	%OMI	75.66 (0.22)^{b}	76.96 (0.33)^{b}	73.73 (0.48)^{a}	<.001
	Crude fibre	%DM	16.31 (0.26)^{b}	14.93 (0.30)^{a}	17.78 (0.46)^{b}	<.001
	NDF	%DM	35.21 (0.54)^{ab}	33.44 (0.51)^{a}	37.94 (0.61)^{b}	<.001
	ADF	%DM	20.05 (0.39)^{b}	18.18 (0.36)^{a}	21.46 (0.54)^{b}	<.001
	CP	%DM	17.47 (0.32)^{b}	15.7 (0.44)a	14.11 (0.39)^{a}	<.001
	Starch	%DM	27.1 (0.75)^{a}	32.59 (0.47)^{b}	27.25 (0.52)^{a}	<.001
	EE	%DM	3.95 (0.21)	3.73 (0.19)	3.42 (0.18)	.174
	NFE	%DM	56.5 (0.45)^{a}	60.64 (0.36)^{b}	58.99 (0.35)^{ab}	<.001
	Ash	%DM	5.6 (0.14)^{b}	4.86 (0.11)^{a}	5.49 (0.18)^{b}	.001
	F/C	%DM	38.83 (1.07)^{a}	36.33 (0.87)^{a}	48.76 (2.29)^{b}	<.001
	NE_L	Mcal/kg DM	1.84 (0.02)^{b}	1.87 (0.01)^{b}	1.75 (0.02)^{a}	<.001
	FE	kg FPCM/kg DMI	1.52 (0.07)	1.61 (0.07)	1.36 (0.08)	.086
	MEI	MJ/d	287.29 (5.44)	298.51 (10.21)	288.18 (10.60)	.715
Feed	SL	%DM	47.66 (3.06)	51.83 (2.81)	45.4 (3.16)	.277
	C-SL	%DM	35.09 (1.63)	39.48 (2.84)	37.1 (2.91)	.351
	HY	%DM	10.61 (2.49)^{ab}	7.32 (1.02)^{a}	21.34 (3.56)^{b}	.003
	S-ML	%DM	10.49 (2.76)	14.04 (2.63)	12.12 (2.51)	.639
	P-ML	%DM	11.59 (2.76)	9.87 (1.91)	4.93 (1.87)	.147
	CT	%DM	17.45 (4.41)	16.1 (3.66)	14.99 (4.33)	.969
	BYP	%DM	1.35 (0.92)	0.29 (0.23)	0.83 (0.56)	.753
	FT	%DM	0.84 (0.57)	0.44 (0.19)	0.38 (0.17)	.873
	AD	%DM	0.00 (0.00)	0.10 (0.08)	0.01 (0.01)	.318

FPCM: fat protein corrected milk; DE: digestible energy; OMI: organic matter intake; dOM: digestible organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; CP: crude protein; EE: ether extract; NFE: nitrogen-free extract; F/C: forage concentrate ratio; NE_L: net energy for lactation; FE: feed efficiency; MEI: metabolisable energy intake; SL: total silage; C-SL: corn silage; HY: hay; S-ML: starch meals; P-ML: protein meals; CT: concentrate; BYP: by-products; FT: fat; AD: additives.
Means within a row with different superscripts (a, b) differ (*p* value $\leq$.05).

Table 6. Enteric methane emissions (means and standard error) expressed as methane production (MeP), methane yield (MeY), methane intensity (MeI) and as percentage of gross energy intake (Ym), solid volatile (VS) excretion, methane emissions from slurry storage and diet GWP in the nutrient clusters (CL-N).

			Cluster			
	Item	Unit	CL-N1	CL-N2	CL-N3	*p* Value
MeP	IPCC	g/d	448.85 (12.78)	455.96 (9.60)	464.55 (19.18)	.811
	IPCC-MCR	g/d	397.41 (20.00)	384.64 (12.31)	433.55 (14.10)	.059
	MIL	g/d	477.77 (4.81)^{ab}	433.56 (13.72)^{a}	485.85 (9.92)^{b}	.030
	MAR	g/d	493.15 (8.77)	501.47 (16.03)	512.22 (17.41)	.914
	KIR	g/d	414.38 (13.15)^{a}	404.30 (13.24)^{a}	470.39 (12.69)^{b}	.004
MeY	IPCC	g/DMI	18.80 (0.49)	18.98 (0.72)	18.55 (0.52)	.781
	IPCC-MCR	g/DMI	16.64 (0.79)	16.13 (0.90)	17.46 (0.82)	.544
	MIL	g/DMI	20.04 (0.34)^{b}	17.84 (0.36)^{a}	19.47 (0.34)^{b}	$<.001$
	MAR	g/DMI	20.65 (0.13)	20.57 (0.14)	20.44 (0.13)	.584
	KIR	g/DMI	17.34 (0.42)	16.67 (0.49)	18.89 (0.64)	.064
MeI	IPCC	g/kg FPCM	12.48 (0.33)^{ab}	11.88 (0.17)^{a}	13.88 (0.65)^{b}	$<.001$
	IPCC-MCR	g/kg FPCM	10.99 (0.38)^{ab}	10.07 (0.38)^{a}	13.16 (0.96)^{b}	.007
	MIL	g/kg FPCM	13.47 (0.76)^{ab}	11.33 (0.39)^{a}	14.82 (1.12)^{b}	.005
	MAR	g/kg FPCM	13.82 (0.62)	13.15 (0.53)	15.46 (0.94)	.104
	KIR	g/kg FPCM	11.60 (0.57)^{ab}	10.66 (0.51)^{a}	14.46 (1.32)^{b}	.020
Ym	Ym-IPCC	% GEI	5.76 (0.04)^{a}	5.79 (0.05)^{a}	5.97 (0.07)^{b}	.028
	Ym-IPCC-MCR	% GEI	5.08 (0.14)	4.90 (0.15)	5.61 (0.20)	.059
	Ym-MIL	% GEI	6.19 (0.23)^{ab}	5.52 (0.17)^{a}	6.32 (0.25)^{b}	.037
	Ym-MAR	% GEI	6.36 (0.17)	6.40 (0.23)	6.62 (0.19)	.570
	Ym-KIR	% GEI	5.34 (0.17)	5.17 (0.20)	6.14 (0.31)	.052
	VS IPCC	kg/d	6.91 (0.23)	6.62 (0.14)	7.35 (0.32)	.132
	CH_4slurry	kg/d	0.32 (0.01)	0.31 (0.01)	0.34 (0.01)	.132
	Diet GWP	kg CO_{2eq}/kg DMI	0.81 (0.03)^{b}	0.78 (0.04)^{ab}	0.63 (0.04)^{a}	.013

MeP: methane production; MeY: methane yield; MeI: methane intensity; Ym: methane conversion factor; IPCC: enteric methane IPCC (Tier 2, 2019); IPCC-MCR: enteric methane IPCC (Tier 2, 2019) with methane conversion ratio from Kirchgessner et al. (1995); MIL: enteric methane from Mills et al.(2003); MAR: enteric methane from Marumo et al. (2023); KIR: enteric methane from Kirchgessner et al. (1995); FPCM: fat protein corrected milk; GEI: gross energy intake; VS: volatile solids; GWP: global warming potential; CO_2eq: carbon dioxide equivalent.

Means within a row with different superscripts (a, b) differ (*p* value $\leq .05$).

emissions were impacted by variations in nutrient composition. Specifically, CL-N2 generally exhibited the lowest MeP, MeY, MeI and Ym. However, significant differences in MeP were observed only in the MIL (433.56 ± 13.72 g/d, $p = .03$) and KIR (403.30 g/d, $p = .004$) models, in MeY only in the MIL model (17.84 ± 0.36 g/kg DMI, $p = .001$), and in MeI across all model predictions except MAR (MIL: 11.33 ± 0.39 g/kg FPCM, $p < .05$). Only IPCC and MIL identified differences considering CH_4 as % of gross energy intake. The same prediction models also confirmed CL-N3 as the highest (MIL: 485.85 ± 9.92 g/d, 19.47 ± 0.34 g/kg DMI, 14.82 ± 1.12 g/kg FPCM, $p < .05$) in terms of CH_4 emissions while the first cluster was average. Considering the diet GWP, the CL-N3 had the lowest (0.63 kg CO_2eq/kg DMI) value meanwhile the CL-N1 the highest (0.81 kg CO_2eq/kg DMI) ($p = .013$). All the data are reported into Table 6.

Discussion

The results presented in this study are specific to lactating Holstein lactating cows fed TMR silage-inclusive diets, without any production specification constraints. The CH_4 emissions estimated across all proxies align with the European averages (Niu et al. 2018; Benaouda et al. 2019). Considerable variability in emission levels has been documented in the literature, influenced by factors such as measurement methods, dietary composition, feed quality, production systems and environmental conditions (de Ondarza et al. 2023). In the Italian context, our estimates align with those found in both *in vivo* and *in vitro* studies for MeY and MeI. However, MeP appears slightly higher, likely due to higher dry matter intake (Pirondini et al. 2012; Colombini et al. 2015; Gislon, Colombini, et al. 2020). This trend of higher estimates in prediction models compared to observed values has been noted before, especially in studies involving Mediterranean diets (Colombini et al. 2023). Conversely, our results are lower than those reported by Bittante et al. in Brown Swiss cows (Bittante et al. 2018). To date, no CH_4 emission factors have been defined for the Italian scenario in general or for specific production contexts. Only Notarnicola et al. (2023) have proposed regional MeP emission factors; however, these are expressed per head without considering the aforementioned variables and are lower than those identified in the current study (Notarnicola et al. 2023).

The positive correlation between MeP, daily milk production and FE was assessed considering equal DMI. Froldi et al. (2024) also demonstrated that farms with greater performance exhibit reduced enteric emissions, which in turn exerts a favourable influence on the carbon footprint of milk (Froldi et al. 2024). MeI was negatively correlated with FE, suggesting that increasing milk production per cow dilutes the maintenance energy requirement per unit of milk produced, thereby reducing CH_4 emissions per unit of milk (Kirchgessner et al. 1995; Capper et al. 2009; Gerber et al. 2013). Huhtanen has identified this as the most sustainable and cost-effective strategy in commercial realities (SLU 2024).

In our study, no significant differences in nutritional parameters and feed composition were observed considering milk productivity, except for C-SL, which was higher in high productivity group, potentially reflecting a higher content of high-quality feed that may contribute to CH_4 emission reductions (Yan et al. 2000).

Interestingly, while IPCC equations suggest a positive correlation between MeP and FE, other models indicate a negative correlation. The relationship between CH_4 emissions and FE remains unclear and warrants further investigation (Nkrumah et al. 2006; Løvendahl et al. 2018; Fregulia et al. 2024). Methane production typically increases with higher feed intake (Blaxter and Clapperton 1965; Robinson et al. 2010); however, it has been demonstrated that an increase of feeding level could reduce MeP due to a faster passage of feed through the rumen, which in turn reduces the time available for microbial fermentation (Yan et al. 2000). In the present study, it was not possible to test the actual digestibility of fibre experimentally, and the use of average data may have limited the capture of variability in the sample.

Considering CH_4 as an energy loss relative to the energy intake, Ym was in line with literature but with a wider range of variability (Yan et al. 2000; Kebreab et al. 2008; Colombini et al. 2023). Nowadays, the IPCC approach is defined as standard to CH_4 estimations; however, the Ym range is rather limited. MIL prediction detected a wider CV of Ym. According to Escobar-Bahamondes et al. (2017), the variability of CH_4 and Ym was greater for models that considered more dietary components as predictors. Empirical models, based on fixed factors like gross energy intake, are more stable and show less variability, but they lack sensitivity to dietary changes that significantly affect CH_4 production. In contrast, mechanistic models, though more complex and data-intensive, offer higher precision by incorporating ruminal dynamics and biochemical pathways (Kebreab et al. 2008). Future research should focus on enhancing the sensitivity of these models to changes in dietary components and feeding strategies, which is essential for accurately predicting the impact of nutrition-based mitigation strategies. Several authors have emphasised the need to develop specific CH_4 emission factors that better capture the specificities associated with dietary choices and improve national inventories and policy-making (Ominski et al. 2007; Alemu et al. 2011; Bannink et al. 2011; Dämmgen et al. 2012; Kouazounde et al. 2015; Eugène et al. 2019; Colombini et al. 2023; Notarnicola et al. 2023).

The proposed clusters, taking into account the type of feed, showed the possible feed choices in terms of combining feeds to meet animals' requirements. However, from a nutritional point of view, they differed in terms of the F:C ratio, without differences in enteric emissions. Decreasing the F:C ratio, increasing concentrates or using silage are factors known to reduce emissions (Beever et al. 1986; Reynolds C et al. 1991; Tamminga et al. 2007; Freetly and Brown-Brandl 2013; Haque 2018). The absence of significant differences in the results obtained may be attributed to the limited variability within the sample population, as all of the diets analysed included silage, with C-SL incorporated at levels exceeding 30%. This composition reflects the typical feeding practices in the Po Valley (Pirondini et al. 2012; Gislon, Bava, et al. 2020; Gislon, Colombini, et al. 2020). Compared to what was shown by Gislon, Bava, et al. (2020), the threshold of $\leq$30% inclusion of C-SL was not confirmed, as at higher inclusion levels the production was higher, although without significant differences.

Clustering by nutrients content identified three different feeding situations. No significant differences in milk production were identified among these groups; however, variations in MeP were observed. This finding indicates that the composition of the diet may already constitute a preliminary impact mitigation strategy. The CN-N3 had higher fibre content and F:C ratio due to a higher HY content and low energy content (NE_L). It is well known that MeP in the rumen is associated with acetic acid production and fermentation of high forage diets results in a higher molar percentage of acetic acid than that obtained with high concentrate diets (Moe and Tyrrell 1979; Holter and Young 1992; Orskov 2012). The clusters also showed differences in emissions, although not all of the equations gave comparable results. Mills equation showed that MeP, MeI and MeY were significantly higher in the CL-N3 cluster, due to a lower starch content and higher crude fibre and ADF fraction. Mills et al. based CH_4 prediction on non-linear model of a modified

Mitscherlich equation with the steepness parameter set to represent the ratio of dietary starch to ADF (Mills et al. 2001, 2003). Holter and Young also reported CH_4 emissions related with forage fraction and used ADF as a predictor (Holter and Young 1992), and several authors confirmed that the use of variable related to content of dietary carbohydrate fractions (cellulose, hemicellulose, starch and sugars) is known to be useful for predicting variation in CH_4 emissions (Moe and Tyrrell 1979; Hindrichsen et al. 2005; Ellis et al. 2009). The fibre effect is also consistent with the KIR results showing differences in MeP as this detailed regression model takes into account several nutrients such as fibre, protein, N-free extracts and ether extracts (EE) and assumes a reducing effect as last one increase. Even if the CL-N3 did not differ in terms of EE, the fibre content for the same DMI resulted in higher emissions. Other empirical models, such as IPCC did not detect differences between nutritional clusters. These models are based on GE intake, and Ym base on DE and NDF. Feed intake is the key factor driving CH_4 emissions and this reflects also the positive correlation between MeP and DMI (Reynolds CK et al. 2011; Ricci et al. 2013). However, the sensitivity of the equation to changes in diet and its characteristics may be limited by considering only DMI or GE (Ellis et al. 2010).

Indeed using of constant Ym may limit the accuracy of the estimate as this factor varies depending on DMI and DM digestibility (Moraes et al. 2014; Appuhamy et al. 2016). In the presented results, the Ym CV was 0.03, meanwhile the MCR of IPCC-MCR model 0.12. Actually, IPCC-MCR emissions were always lower than IPCC and this model tended to detect differences among emission. Clear differences were found between the emissions when the dietary nutrient content was considered and the emissions expressed as MeI. These were also significant with the IPCC equations. The CL-N2 diet cluster was found to have a higher energy content and protein content than the CL-N3 diet cluster. However, the CL-N2 diet cluster also had a lower crude fibre and ADF content. This cluster showed also an average higher FPCM, and it can be hypothesised that these results are attributable to the higher energy density that is available for milk production and the maintenance of an adequate supply of fibre, which is essential for rumination processes and the regulation of ruminal transit rate. The diets quality has been reported to reduce CH_4 intensity by increasing milk production per cow, diluting the amount of feed required per unit of milk, and/or altering rumen fermentation conditions (Dong et al. 2004; Knapp et al. 2014).

The ration composition also obviously influences nutrient excretion, and in particular the excretion of VSs affects what may be the CH_4 emissions from the effluent during storage (Aguerre et al. 2011; Appuhamy et al. 2014). Even when considering nutrient clusters, no differences in terms of VS were observed. This result appears to contradict the findings of Appuhamy et al. (2018), which suggest that improving the nutritional quality of the diet, particularly the digestibility of organic matter, influences VS excretion. This may be due to the fact that in our research we have not real data related to the diet digestibility and we are not able to appreciate the differences between the clusters.

When considering the GWP of feed clusters showed no significant differences. GWP values ranged from 0.45 to 1.17 kg CO_2eq/kg DMI, with most values ranging between 0.61 and 0.77, consistent with findings by Gislon, Bava, et al. (2020) and, to the best of our knowledge, no other study has investigated the same aspect. The variability between the clusters depends on the type of feed included and its GWP. CL-F2 had higher values compared to the other clusters (0.83), which can be attributed to its greater inclusion of CTs containing more protein sources, such as soybean meal. In terms of nutritional quality, the best cluster had an intermediate GWP value of 0.78 CO2eq/kg DMI, attributed to the high content of home-grown feeds, such as silage and maize silage, which can provide fibre and starch with a low production GWP. Conversely, the lower quality cluster (CL-N3) showed the lower GWP value due to a higher inclusion of HY, CT and P-ML.

Conclusions

This research highlights that feed quality – specifically higher starch content and lower ADF levels – significantly reduces enteric CH_4 emissions while simultaneously improving milk production. In this production context, the type of feed used did not directly influence total emissions or the GWP of the diet; instead, the nutritional quality of the ration played a crucial role. Rations with elevated starch and reduced fibre contents enhanced animal performance and decreased CH_4 energy losses (MeI), underlining the importance of balanced and high-quality diets as a key mitigation strategy.

Evaluating emissions in terms of intensity rather than absolute production emerged as a promising approach, aligning better with improvements in FE and sustainability goals.

The choice of CH_4 prediction model also influenced the assessment of feeding strategies. In particular, the Mills equation showed greater sensitivity to dietary variations compared to empirical models based solely on intake variables, suggesting the necessity of developing context-specific emission factors that better capture differences in diet composition and production systems.

Future research should focus on refining CH_4 prediction algorithms by integrating key nutritional parameters and designing cost-effective optimisation models that consider both emission reduction and productivity targets. Additionally, prioritising strategies based on MeI rather than total output could accelerate sustainability transitions in dairy farming, particularly in silage-based systems common to Mediterranean climates. Broader validation across diverse production settings will be essential to fully extend these findings.

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Data availability statement

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

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