Methane emissions from manure of dairy cows

influence of feed efficiency and dietary composition

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Methane emissions from manure of dairy cows-influence of feed efficiency and dietary composition

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Abstract

Methane (CH_4) is a strong greenhouse gas (GHG) linked to climate change, and dairy farms are a key source of these emissions. Most studies look at enteric CH_4 from digestion, but manure stored on farms also adds to the total emissions. Feed efficiency (FE) is the ability of an animal to convert feed into productive output (e.g., milk) and is commonly measured using residual feed intake (RFI), which is defined as the difference between an animal's actual and its predicted feed intake based on maintenance and production requirements. More efficient animals are typically consuming less feed for a given level of production, although this relationship is not always consistent. Differences in FE and diet may influence the quantity and composition of excreted organic matter (OM), which can affect CH_4 production during manure fermentation. This study looked at how FE and diet affect CH_4 from dairy cow manure.

A total of 16 dairy cows were used in a 2×2 factorial design in a previous study, differing in FE classified as efficient (E) or inefficient (INE), and diets differed in forage-to-concentrate (F:C) ratio, with treatments of 55:45 (F55) and 70:30 (F70). Manure samples were collected from all cows and were incubated at 22 °C in the lab.

The results showed that CH_4 production from manure was not significantly affected by FE or diet composition. Although manure from feed-efficient cows produced more total gas, CH_4 production remained similar across treatments. Other fermentation parameters, including pH, dry matter (DM) disappearance, and volatile fatty acids (VFA), were also comparable among groups.

In conclusion, FE and diet composition did not affect CH_4 production from dairy cow manure. In a related experiment conducted with the same animals, efficient cows produced less enteric CH_4, whereas manure CH_4 production remained unchanged across efficiency groups in the present study. These findings suggest that improving FE can reduce enteric CH_4 emissions without increasing emissions from manure, indicating no trade-off between enteric and manure CH_4 production.

These findings also suggest that changes in diet alone may not be sufficient to reduce CH_4 emissions from manure. Instead, improving manure management practices may be an effective approach for reducing overall CH_4 emissions from dairy production systems.

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Abbreviations

Abbreviation	Description
CH_4	Methane
CO_2	Carbon dioxide
DM	Dry matter
E	Feed efficient
FAO	Food and Agricultural Organisation
F:C	Forage-to-concentrate
FE	Feed efficiency
FSM	Faeces-slurry mixture
F55	Diet containing 55% forage and 45% concentrate
F70	Diet containing 70% forage and 30% concentrate
GC	Gas chromatograph
GHG	Greenhouse gas
H_2	Hydrogen
IEA	International Energy Agency
INE	Inefficient
IPCC	Intergovernmental Panel on Climate Change
mL	Millilitre
Mmol	Millimole
OM	Organic matter
RFI	Residual feed intake
TGP	Total gas production
VFA	Volatile fatty acids
VS	Volatile solids

1. Introduction

1.1 Background and Literature review

Methane (CH_4) is a highly potent greenhouse gas (GHG), with a global warming potential estimated to be 28–36 times greater than that of carbon dioxide (CO_2) over a 100-year time horizon. When evaluated over a shorter 20-year period, its warming potential increases substantially to approximately 84–87 times that of CO_2, highlighting its significant impact on near-term climate change (IEA 2021). Due to this high radiative efficiency, CH_4 is considered a critical target for short-term climate mitigation strategies.

Globally, approximately 60% of CH_4 emissions originate from anthropogenic activities. The major sources include fossil fuel production and use, agriculture, waste management, and industrial processes. Among these, the oil and gas sector is a dominant contributor, with emissions arising from extraction, processing, and distribution, accounting for more than 120 million tonnes of CH_4 annually (IEA 2021).

Agricultural sources are projected to emit 3,135.75 Mt CO_2-eq of CH_4 annually, making the sector the largest contributor to anthropogenic CH_4 emissions. In fact, agricultural activities account for 50.6% of total human-related CH_4 emissions, with enteric fermentation representing 59.8% of these emissions, followed by rice cultivation, other agricultural practices, and manure management (Karakurt et al. 2012).

Within the livestock sector, dairy cattle are particularly important contributors due to their large global population, high feed intake, and continuous production cycles (Herrero et al. 2016; FAO 2019). Methane emissions from dairy production arise from two main sources: enteric fermentation in the rumen and anaerobic decomposition of manure during storage and handling.

Enteric CH_4 is produced during the microbial fermentation of feed in the rumen. The rumen is a highly specialised anaerobic fermentation chamber in ruminants

where a complex microbial ecosystem degrades ingested feed into absorbable nutrients. Structural carbohydrates such as cellulose and hemicellulose are hydrolysed into simple sugars and subsequently fermented into volatile fatty acids (VFA), microbial biomass, and gases like CO_2, and hydrogen (H_2). The main VFA - acetate, propionate, and butyrate are absorbed across the rumen wall and provide up to 70% of the animal's metabolizable energy (Bergman 1990).

Methanogenic archaea utilize H_2 to reduce CO_2, forming CH_4 as a metabolic by-product (Moss et al. 2000). This process is essential for maintaining rumen function by preventing H_2 accumulation, which would otherwise inhibit microbial metabolism (Hook et al. 2010; Janssen 2010). However, CH_4 production represents an energetic inefficiency, resulting in a loss of up to 12% of the animal's gross energy intake (Giger-Reverdin & Sauvant 2000).

Alternative pathways for H_2 utilisation can compete with methanogenesis and thereby reduce CH_4 emissions. Among these, propionate formation is the most significant. During this process, H_2 is incorporated into propionate, reducing its availability for CH_4 production (Ungerfeld 2015).

Other pathways include reductive acetogenesis, nitrate reduction, and sulfate reduction. Although reductive acetogenesis converts H_2 and CO_2 into acetate, it is typically less competitive than methanogenesis in the rumen environment. Nitrate and sulfate reduction pathways can act as effective H_2 sinks but are limited by risks of toxicity, such as nitrate poisoning (Olijhoek et al. 2016).

1.1.1 Methane from manure

In addition to enteric emissions, CH_4 is produced during manure storage under anaerobic conditions, where methanogenic archaea convert organic matter (OM) and intermediates such as H_2 and CO_2 into CH_4. The extent of CH_4 production from manure depends on several factors, including the amount of volatile solids (VS), storage conditions (e.g., temperature and retention time), and manure management practices (Steed & Hashimoto 1994; Sommer et al. 2007). Although manure-derived CH_4 emissions are generally lower than enteric emissions, they represent a

significant component of total emissions from dairy systems and should not be overlooked (IPCC 2019).

Diet is a key factor influencing manure composition and its CH_4 producing potential. Feed composition affects digestion and determines the amount and type of OM excreted. Methane production from manure generally increases with higher feed intake or digestibility due to greater faecal output; however, highly digestible diets may leave less fermentable material in manure, reducing CH_4 per unit of faeces (Huhtanen et al. 2021).

The type of dietary carbohydrates also plays an important role. Forage-based diets contain higher levels of structural carbohydrates, which are less digestible and can increase fibre excretion, thereby enhancing CH_4 production during storage (Hindrichsen et al. 2005). In contrast, concentrate-rich diets provide readily fermentable carbohydrates such as starch and sugars, which promote propionate formation in the rumen and reduce CH_4 emissions (Martin et al. 2010).

Dietary fat supplementation can further influence CH_4 emissions by reducing fibre degradation and inhibiting methanogenic microorganisms, potentially lowering the amount of fermentable substrate in manure (Johnson & Johnson 1995).

While enteric CH_4 emissions have been extensively studied, fewer studies have examined how these factors affect CH_4 production from manure. Recent research has highlighted the importance of considering manure as an integral part of CH_4 mitigation strategies. For example, Hassanat & Benchaar (2019) demonstrated that dietary factors influencing rumen fermentation can also affect CH_4 emissions from stored manure, indicating a strong linkage between processes occurring within the animal and those occurring during manure storage. This highlights the need for a systems-level approach when evaluating CH_4 mitigation strategies in dairy production.

1.1.2 Feed Efficiency and CH_4 Emissions

Feed efficiency is a central concept in dairy production, influencing both economic profitability and environmental sustainability. It reflects the ability of an

animal to convert feed into productive outputs such as milk. Improving FE reduces feed costs and decreases resource use, thereby lowering the environmental footprint of dairy systems (Løvendahl et al. 2018).

Residual feed intake is widely used as a measure of FE. It is defined as the difference between an animal's actual feed intake and its predicted intake based on maintenance and production requirements (Arthur & Herd 2008). Animals with negative RFI values are considered more efficient because they consume less feed than expected for a given level of production (De La Torre et al. 2019). Importantly, RFI is independent of body size and milk yield, making it a valuable trait for comparing animals under similar production conditions (Herd & Arthur 2009).

Variation in FE is associated with differences in digestion, metabolic efficiency, activity levels, and nutrient partitioning (Herd et al. 2004). These factors can also influence CH_4 production, which is closely linked to feed intake and rumen fermentation processes. Several studies have reported that more feed-efficient animals tend to produce less enteric CH_4 per unit of feed intake or milk produced (Nkrumah et al. 2006; Hegarty et al. 2007) potentially due to improved digestion, faster passage rates, or shifts in rumen microbial populations (Herd et al. 2004).

While the relationship between FE and enteric CH_4 emissions has been relatively well studied, much less is known about how FE influences CH_4 production from manure. Feed-efficient animals are likely to excrete less undigested OM due to improved nutrient utilization. As a result, manure from these animals may contain fewer fermentable substrates, potentially reducing CH_4 production during storage (Huhtanen et al. 2021).

Furthermore, the interaction between FE and other factors, such as diet composition, adds complexity to the system. Understanding how these factors interact is essential for developing effective mitigation strategies that consider both animal performance and environmental impact (Gerber et al. 2013).

1.1.3 Effect of Diet on CH_4 Emissions

Diet composition is one of the most important and practical factors influencing CH_4 emissions in dairy systems. It affects rumen fermentation pathways, nutrient digestibility, and the quantity and composition of OM excreted in manure (Knapp et al. 2014).

The forage-to-concentrate (F:C) ratio in the diet plays a key role in determining CH_4 production. Forage-based diets are rich in structural carbohydrates such as cellulose and hemicellulose, which promote acetate formation during rumen fermentation. This process generates H_2, which is subsequently used for CH_4 production, resulting in higher CH_4 emissions (Beauchemin et al. 2009; Knapp et al. 2014). In contrast, concentrate-rich diets increase starch availability and promote propionate formation, which acts as a H_2 sink and reduces CH_4 production (Beauchemin et al. 2009; Ungerfeld 2015).

Dietary fat supplementation has been shown to reduce CH_4 emissions. A meta-analysis by Grainger & Beauchemin (2011) demonstrated a linear relationship between dietary fat content and CH_4 yield, with a 10 g/kg increase in fat reducing CH_4 yield by approximately 1 g/kg DM intake in cattle within a practical feeding range (<80 g/kg DM). This indicates that lipid supplementation can be an effective mitigation strategy; however, fat inclusion must remain within practical limits, as higher levels can affect rumen function and animal performance (Enjalbert et al. 2017).

Recent studies have demonstrated that dietary manipulation affects not only enteric CH_4 emissions but also CH_4 production from manure. The effects of dietary lipid supplementation on CH_4 emissions from stored manure appear to be inconsistent across studies. For instance, supplementation with rapeseed oil has been shown to reduce manure CH_4 emissions (Ramin et al. 2021). In contrast, studies by Hassanat & Benchaar (2019) reported increased CH_4 production from manure following lipid supplementation with linseed oil. These contrasting findings suggest that the impact of dietary oils on manure CH_4 emissions is variable and likely influenced by factors such as oil type, inclusion level, diet composition,

and manure characteristics. These findings underscore the importance of adopting a holistic approach when evaluating CH_4 mitigation strategies in dairy systems.

1.1.4 Methane Measurement and Modelling Approaches

Accurate measurement of CH_4 emissions is essential for understanding the effects of diet and FE. *In vitro* techniques have become increasingly important tools for studying CH_4 production under controlled conditions.

Ramin & Huhtanen (2012) developed a fully automated *in vitro* gas production system to measure CH_4 production kinetics. This system allows for detailed analysis of fermentation processes and provides valuable insights into how different substrates influence CH_4 production.

More recently, Vadroňová et al. (2026) demonstrated that CH_4 emissions can be effectively modelled based on diet composition, particularly the F:C ratios and retention times. These modelling approaches are useful for predicting CH_4 production and evaluating mitigation strategies without the need for large-scale *in vivo* experiments.

In vitro methods are particularly valuable for studying CH_4 production from manure, as they allow for controlled simulation of storage conditions and detailed assessment of fermentation dynamics. This makes them highly relevant for evaluating CH_4-producing potential of manure from animals fed different diets.

1.1.5 Knowledge Gap

Despite significant progress in understanding CH_4 emissions from dairy systems, important gaps remain. Most research has focused on enteric CH_4 emissions, while CH_4 production from manure has received comparatively less attention.

Although recent studies have shown that diet composition influences manure CH_4 emissions, the role of FE in this context is not well understood. In particular, there is limited information on how FE and diet interact to influence CH_4 production from manure.

Furthermore, few studies have combined *in vitro* approaches with animal-level data to evaluate CH_4 emissions from manure. Addressing these gaps is essential for developing integrated mitigation strategies that consider the entire production system, from feed intake to manure management.

2. Mitigation Strategies for Manure CH_4 Production

Methane emissions from manure can be reduced through a combination of management practices, dietary strategies, and technological interventions. Since CH_4 is produced under anaerobic conditions during manure storage, most mitigation approaches aim to either reduce the amount of degradable OM in manure or limit the conditions that favour methanogenesis (Montes et al. 2013).

One of the most effective strategies is improving manure management. Ambrose et al. (2023) reported that stored liquid manure is a major source of CH_4 emissions. They also noted that practices such as frequent removal of manure can help reduce emissions, showing that storage conditions play an important role in CH_4 production. In addition, solid manure systems generally produce less CH_4 compared to liquid systems because they allow more oxygen penetration, which suppresses methanogenic activity (Sommer et al. 2007; IPCC 2019).

Another important approach is anaerobic digestion (biogas production). In this system, manure is intentionally digested under controlled anaerobic conditions to capture CH_4 as biogas, which can then be used as a renewable energy source. This process not only reduces CH_4 emissions from storage but also contributes to energy production and nutrient recycling (Gerber et al. 2013).

Dietary manipulation is also an indirect but important mitigation strategy. Diets that improve digestibility reduce the amount of undigested OM excreted in manure, thereby lowering the substrate available for CH_4 production (Haque 2018). For example, increasing the proportion of concentrates or improving forage quality can reduce CH_4 potential in manure. Similarly, dietary fat supplementation has been shown to reduce CH_4 emissions by altering digestion and reducing fermentable substrates in manure (Beauchemin et al. 2008).

Feed efficiency also contributes to mitigation. More feed-efficient animals are generally characterized by improved nutrient utilization, which may influence the quantity and composition of OM excreted (Herd et al. 2004; Berry & Crowley 2013). As a result, manure from efficient cows may produce less CH_4 during storage

compared to manure from less efficient animals. This highlights the potential of selecting animals with improved FE as a long-term mitigation strategy (Lucas et al. 2026).

Finally, manure treatment methods such as composting or aerobic treatment can reduce CH_4 emissions by introducing oxygen, which inhibits methanogenic microorganisms. However, these systems must be carefully managed, as they may increase emissions of other gases such as nitrous oxide (He et al. 2001).

2.1 Aim and Hypotheses

The aim of this study was to evaluate CH_4 emissions from manure of dairy cows differing in FE and fed different diets. It was hypothesized that emissions differ between feed-efficient and feed-inefficient cows, are influenced by diet composition, and are affected by an interaction between FE and diet.

3. Materials and Methods

3.1 Experimental Design, Sample Collection and Preparation

The study was conducted to evaluate the effects of FE status and dietary F:C ratio on manure fermentation characteristics using an *in vitro* incubation approach. The experimental design followed a 2 × 2 factorial arrangement, using 16 dairy cows from a previous study, with four animals assigned to each treatment group with FE status classified as efficient (E) or inefficient (INE), and diets differed in F:C ratio, with treatments of 55:45 (F55) and 70:30 (F70) as the main factors.

All cows were fed diets based on grass silage consisting primarily of timothy (*Phleum pratense*) and red clover (*Trifolium pratense*). The concentrate portion included commercially formulated feeds, Komplett Fibre 165 (2024) and Mixa Intensiv MG (2023). While feed ingredients remained consistent across treatments, the F:C ratio varied according to the experimental design.

Faecal samples were collected from each cow at six time points over three consecutive days to account for diurnal variation. Samples from each animal were pooled to form a representative composite sample. Following collection, samples were stored at –20 °C until further processing.

Slurry used as inoculum was collected on the morning of the first experimental day from a depth of at least 50 cm to ensure anaerobic conditions and avoid oxygen exposure.

Faecal and slurry samples were collected and transported to the laboratory for further processing. The slurry was first filtered, and its pH was measured before being aliquoted and stored until use. Faeces-slurry mixture (FSM) was then prepared in a 2:1 ratio by combining 800 g of faeces with 400 g of slurry. The mixture was thoroughly homogenised while maintaining a continuous flow of CO_2 to minimise oxygen exposure and preserve anaerobic conditions.

3.2 *In vitro* incubation procedure

At the beginning of the incubation, approximately 300 g of the FSM was added to pre-weighed and tared serum bottles (500 mL). Each bottle was flushed with CO_2 both before and after filling to ensure anaerobic conditions were maintained. To determine the primary DM content, around 400 g of the FSM was placed into pre-weighed aluminium trays (one per cow), oven dried at 60 °C for 48 hours and then reweighed. The primary DM percentage was calculated as the ratio of dry weight to wet weight. The procedure was repeated for the remaining samples.

$$\text{Primary DM\%} = (\text{Dry weight}/\text{Wet weight}) \times 100$$

Gas measurements were carried out throughout the incubation period to monitor CH_4 production and total gas production (TGP) according to the method described by Ramin et al. (2021). Measurements were taken on days 1, 4, 7, 11, 14, 21, 28, 35, and 42. At each sampling point, CH_4 concentration was measured prior to total gas volume. A gas sample of approximately 0.6 mL was withdrawn from the bottle headspace of each bottle using a gas-tight syringe (Hamilton, Bonaduz, Switzerland) through a rubber Suba-seal septum (Z124567-100EA, 13, Sigma-Aldrich). Before sampling, the gas in the bottle headspace was gently mixed by moving the syringe plunger in and out several times. From this 0.6 mL gas sample, a 0.2 mL aliquot was injected into a gas chromatograph (GC) (Thermo Scientific TRACE 1300^{TM} Series GC, Thermo Fisher Scientific S.p.A. Milan, Italy) equipped with a thermal conductivity detector for CH_4 analysis (Figure 1A). After sampling, the septum was resealed with Blu Tack (Bostik, Leicester, UK) to maintain airtight conditions.

Following CH_4 sampling, the TGP was measured by releasing the gas from each bottle into a gas-tight inert foil sampling bag (Supel ^{TM} inert foil gas sampling bag screw cap valve, 0.6 litres, Bellefonte, USA) connected to the serum bottle via a valve system (Figure 1B). The volume of gas was then determined using a 100 mL syringe (BD Plastipak ^{TM}, Becton, Dickinson and Company, Franklin Lakes, USA), which was then connected to the bag to withdraw the gas, which was subsequently used to quantify the total gas volume. Methane volume (mL) at each sampling time

point was calculated by accounting for both CH_4 present in the bottle headspace and CH_4 released in the collected gas. The calculation was based on the measured CH_4 concentration (CH_4 %) and the total gas volume produced, as shown in the following equation:

CH_4 (mL) = (CH_4 concentration × headspace volume) + (CH_4 concentration × gas volume produced)

where CH_4 (mL) is the total CH_4 production, CH_4 concentration is the CH_4 concentration in sampled gas, headspace volume represents the gas volume in the bottle headspace, and gas volume produced corresponds to the cumulative gas generated during incubation, according to the method described by Ramin et al. (2021).

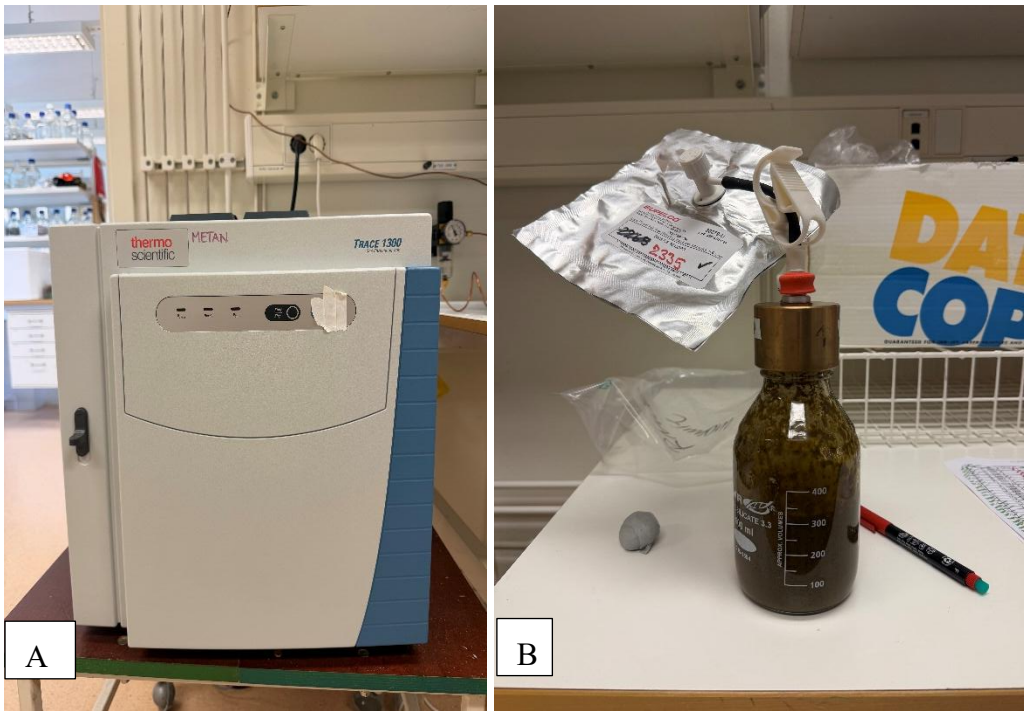

Figure 1.A) Gas chromatograph (Thermo Scientific Trace 1300) for measurement of CH4 produced. B) An incubation bottle containing manure slurry connected to a gas-tight foil sampling bag via a valve system for collection of produced gas. (Photo Credits: Vasudev)

3.3 Termination of Incubation and Post-Incubation Analyses

At the end of the incubation period, each bottle was carefully removed and handled to avoid any loss of material or gas. The total weight of each bottle,

including its contents, was recorded to assess any changes during incubation. Immediately after opening the bottles, the pH of the incubated material was measured using a pH meter to evaluate the extent of microbial fermentation and acidification.

Following pH measurement, pre-weighed aluminium trays were prepared for each sample. Each tray was properly labelled with the corresponding cow identification number, replicate (A or B), and marked as residue (R) to ensure accurate sample tracking. The empty trays were weighed and tared prior to use.

The contents of each incubation bottle were then quantitatively transferred into the respective aluminium trays. Care was taken to empty the bottles as completely as possible to minimize sample loss and ensure accurate determination of residual mass. After transfer, the combined weight of the tray and sample was recorded. A sample of 1.5 grams from the residue is collected by squeezing and stored in Eppendorf tubes at –18 °C for VFA analysis.

The trays containing the incubation residues were then placed in a drying oven set at 105 °C for 16 hours. This drying step allowed for the removal of moisture and enabled the determination of the secondary DM content. After drying, the trays were removed from the oven and subsequently weighed.

The final dry weight obtained after drying was used to calculate the secondary DM content of the incubated material. This parameter reflects the remaining undigested fraction after the incubation period and provides insight into the extent of OM degradation during anaerobic fermentation.

3.4 Volatile fatty acids analysis

After termination of the incubation, a 1.5 g of liquid squeezed from the FSM residue was collected into Eppendorf tubes and stored at −18 °C until further analysis. On the day of VFA analysis, samples were thawed at room temperature and centrifuged at 12,000 rpm for 10 minutes using a Sigma 2K15 laboratory centrifuge (Figure 2A) to separate solid particles.

The supernatant was collected and prepared for analysis using a 5× dilution. For each sample, 200 µL of centrifuged FSM fluid was mixed with 660 µL of distilled water, 100 µL of internal standard solution, and 40 µL of 25% Phosphoric acid were added to a 2 mL GC autosampler vial and the solution was vigorously shaken for 5 s by a vortex shaker and analysed using a GC equipped with a Thermo Scientific AI 1310 Autosampler (Figure 2B).

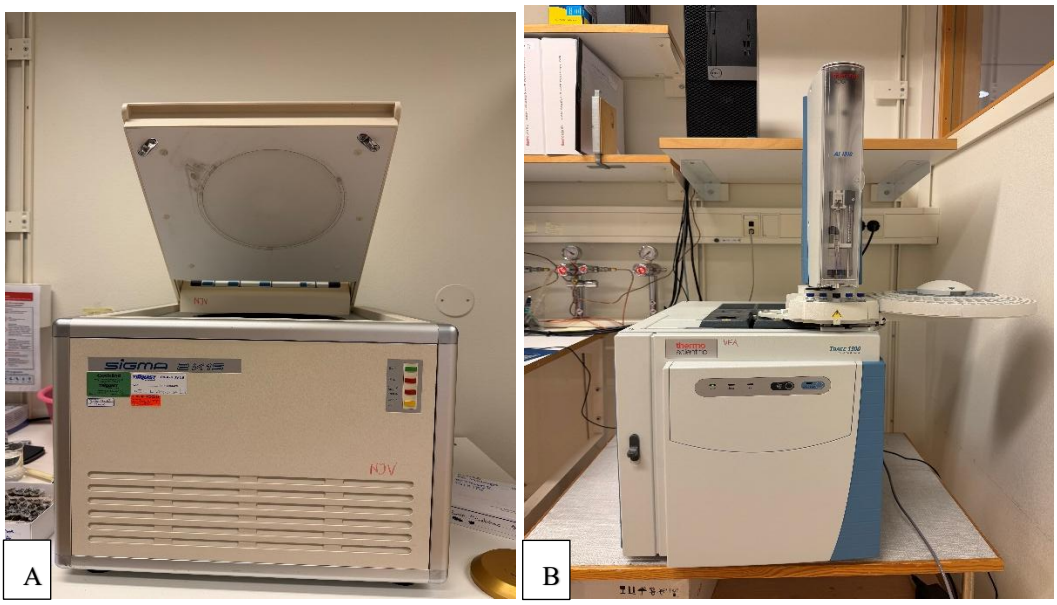

Figure 2.(A) Sigma 2K15 laboratory centrifuge used for sample separation prior to analysis; (B) Thermo Scientific AI 1310 autosampler used for VFA analysis. (Photo Credits: Vasudev)

3.5 Statistical analysis

Data was analyzed using a linear model in SAS (PROC MIXED; SAS Institute Inc., Cary, NC, USA). The model included efficiency (efficient vs. inefficient), diet (F55 vs. F70), and their interaction as fixed effects:

$$Y_{ijk} = \mu + \text{Eff}_i + \text{Diet}_j + (\text{Eff} \times \text{Diet})_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} represents the response variable, μ is the overall mean, Eff_j is the fixed effect of efficiency, $Diet_j$ is the fixed effect of diet, $(Eff \times Diet)_{ij}$ is their interaction, and ε_{ijk} is the residual error.

Technical replicates (duplicate bottles) were averaged prior to analysis so that the experimental unit was the individual cow (n = 4 per treatment combination; total n = 16). Some bottles developed mold and were therefore excluded from the final data analysis. Least squares means were compared using pairwise differences, and significance was declared at $P < 0.05$.

4. Results

4.1 Methane, TGP, pH and DM disappearance

The effects of FE and diet on CH_4 production, TGP, pH, and DM disappearance are presented in Table 1.

Methane production, expressed both as total volume (mL) and per unit of DM (mL/g DM), was not significantly affected by FE phenotype, diet, or their interaction ($P > 0.05$). Numerically, CH_4 production (mL) was higher in efficient animals compared to inefficient animals that were fed with the F55 diet (57.6 vs. 42.9 mL), while values were similar under the F70 diet (56.8 vs. 55.6 mL). A similar pattern was observed for CH_4 expressed per unit of DM. All CH_4 values presented in the Figure 3 were blank corrected.

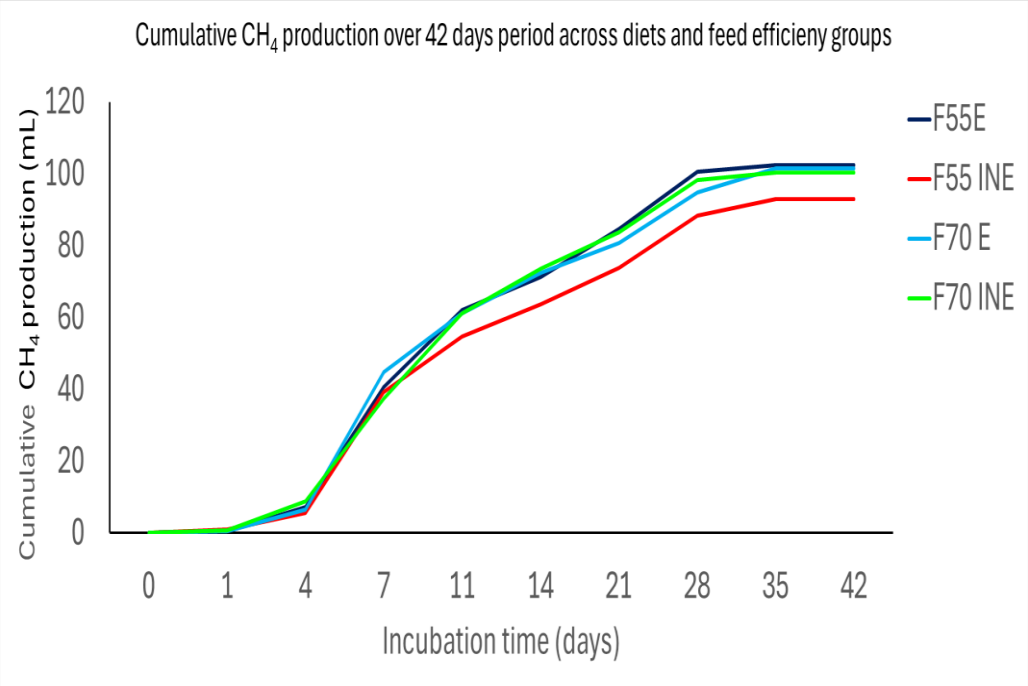

Figure 3.Cumulative CH4 production (group average) over time for four treatments (F55 = forage-to-concentrate ratio 55:45; F70 = forage-to-concentrate ratio 70:30; E = Efficient (E), INE = Inefficient).

Total gas production (mL) was significantly affected by FE (P = 0.019), with efficient animals producing more gas than inefficient animals. Under the F55 diet, efficient animals produced substantially higher gas volumes than inefficient animals (305 vs. 130 mL), while under the F70 diet, the difference was smaller (332 vs. 242 mL). No significant effects of diet or interaction between diet and efficiency were observed for TGP. When expressed per unit of DM (mL/g DM), TGP did not differ significantly among treatments (P > 0.05), although efficient animals showed slightly higher values. All TGP values presented in the Figure 4 were blank corrected.

The pH values at the end of incubation were similar across all treatments and were not affected by FE, diet, or their interaction (P > 0.05), remaining within a narrow range (6.12–6.17).

Dry matter disappearance (%) was also not significantly influenced by FE, diet, or their interaction (P > 0.05), with values being consistent across all treatments (approximately 67%).

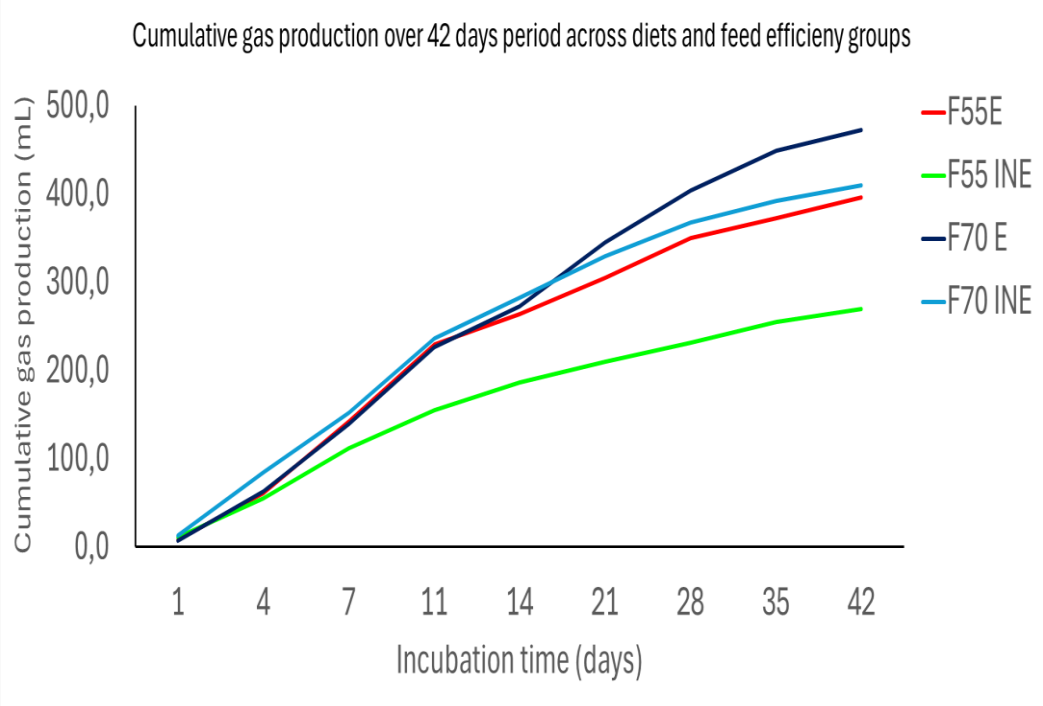

Figure 4.. Cumulative gas production (group average) over time for four treatments (F55 = forage-to-concentrate ratio 55:45; F70 = forage-to-concentrate ratio 70:30; E = Efficient (E), INE = Inefficient).

Table 1. CH_4, Total gas production, pH and DM disappearance in efficient and inefficient groups under F55 and F70 diets

Item	Efficient		Inefficient			P-Value		
	F55	F70	F55	F70	SEM	Eff	Diet	Eff×Diet
CH_4 mL	57.6	56.8	42.9	55.6	6.70	0.25	0.39	0.33
CH_4 (mL/g DM)	1.67	2.06	1.42	1.77	0.23	0.28	0.15	0.93
Total gas mL	305	332	130	242	38.4	0.019	0.16	0.38
Total gas (mL/g DM)	7.93	9.15	5.15	7.66	1.10	0.13	0.18	0.61
pH	6.15	6.12	6.17	6.15	0.072	0.73	0.73	1.00
DM disappearance %	67.4	67.2	67.2	67.3	0.37	0.98	0.90	0.76

F55 = forage-to-concentrate ratio 55:45; F70 = forage-to-concentrate ratio 70:30; SEM = standard error of the mean.

4.2 Volatile Fatty Acids

The results for VFA concentrations and proportions are presented in Table 2. No significant effects of FE, diet, or their interaction were observed for any of the measured VFA parameters ($P > 0.05$).

Acetic acid concentrations were similar across all treatments. Butyric acid showed slightly higher values in animals fed the F70 diet compared to F55, but the differences were not significant. Propionic acid values were also similar among all groups. Total VFA concentration did not differ between treatments and remained within a narrow range across all groups.

For the minor VFA components (valeric, isovaleric, and isobutyric acids), no significant differences were observed. However, some small numerical differences were seen, especially in inefficient animals fed the F70 diet, which showed slightly higher values for some of these acids.

Table 2. Volatile fatty acid concentrations and molar proportions in Efficient and Inefficient Groups under F55 and F70 Diets

Item	Efficient		Inefficient			P-Value		
	F55	F70	F55	F70	SEM	Eff	Diet	Eff×Diet
Total VFA mmol/g DM	0.70	0.65	0.69	0.60	0.103	0.807	0.497	0.825
Acetic acid mmol/g DM	421	368	420	368	41.0	0.998	0.224	0.988
Butyric acid mmol/g DM	311	373	327	349	41.8	0.927	0.337	0.644
Propionic acid mmol/g DM	207	198	190	209	13.3	0.819	0.714	0.301
Valeric acid mmol/mol	23.5	27.3	26.4	26.6	2.41	0.670	0.418	0.475
Isovaleric acid mmol/mol	18.7	16.6	18.1	23.4	2.49	0.242	0.517	0.157
Isobutyric acid mmol/mol	16.7	16.0	16.9	21.8	2.11	0.184	0.345	0.205

F55 = forage-to-concentrate ratio 55:45; F70 = forage-to-concentrate ratio 70:30; SEM = standard error of the mean.

5. Discussion

The present study evaluated the effects of FE, diet composition, and their interaction on CH_4 production from dairy cow manure under controlled *in vitro* conditions. Methane production was not significantly affected by any of the tested factors, TGP was higher in manure from efficient animals, and pH, DM disappearance, and VFA profiles remained stable. These findings indicate that manure methanogenesis was relatively insensitive to moderate variation in the F:C ratio and FE under the applied conditions.

5.1 Methane production

Manure CH_4 production was not affected by the F:C ratio or FE. Increasing the proportion of forage typically promotes acetate production and enhances H_2 availability, resulting in increased CH_4 formation, whereas higher concentrate inclusion often shifts fermentation toward propionate and reduces methanogenesis (Beauchemin et al. 2009; Hristov et al. 2013; Knapp et al. 2014).

However, the absence of a dietary effect in the present study suggests that rumen-based CH_4 responses did not translate into measurable differences in manure methanogenesis. This is consistent with evidence that dietary effects on CH_4 emissions are often more pronounced for enteric fermentation than for manure. For instance, Ramin et al. (2021) observed a strong reduction in enteric CH_4 emissions (on average 473 vs. 607 L/d) when diets were supplemented with rapeseed oil, while only a modest, non-significant decrease in faecal CH_4 production (on average 3.45 L/kg DM vs. 3.84 L/kg DM) was detected, indicating that dietary impacts on manure CH_4 may be limited or less consistent. Møller et al. (2014) demonstrated that diet can influence manure CH_4 potential primarily when it substantially alters faecal composition, particularly the content and degradability of VS. In that study, dietary fat levels ranged from approximately 3.7% to 6.4% of DM and were increased using rapeseed-based supplements increased CH_4 yield from manure by 25–31%, highlighting the importance of substrate characteristics rather than fermentation intensity alone.

In contrast, other studies have found clear diet effects on CH_4 from manure when the diet differences were larger. Hassanat & Benchaar (2019) showed that manure from cows fed corn silage produced substantially more CH_4 than manure from cows fed red clover silage, with maximum CH_4 production potentials of 182 and 118 L CH_4/kg VS, respectively, representing a 54% increase with corn silage. In addition, supplementation with linseed oil at 4% of diet DM further increased CH_4 production potential by 17%. These differences were associated with greater VS degradation in manure from corn silage diets (30.6% vs. 22.5%), highlighting that both forage type and dietary fat supplementation can significantly influence substrate degradability and CH_4 production during manure storage. Similarly, Benchaar & Hassanat (2019) found that manure from cows fed brown midrib corn silage produced more CH_4 (about 220–250 g CH_4/kg VS) than manure from cows fed conventional corn silage (about 180–200 g CH_4/kg VS). This is likely because these diets produced more easily degradable material in the manure. Hindrichsen et al. (2005) showed that carbohydrate composition, including lignified and non-lignified fibre, pectin, fructan, sugar, and starch, affected CH_4 emissions from both cows and their slurry, even at a constant F:C ratio (1:1). Diets rich in fructan resulted in higher digestibility and CH_4 production, whereas lignified fibre reduced CH_4 emissions. Methane from slurry accounted for 16.0–21.9% of total emissions, indicating that carbohydrate type influences both enteric and manure CH_4 through its effect on substrate degradability.

The absence of a FE effect on manure CH_4 production aligns with evidence that the mechanisms linking FE to CH_4 emissions operate primarily at the level of feed intake, digestion, and rumen fermentation, which governs the enteric CH_4 formation, whereas CH_4 from manure is generated during anaerobic degradation of excreted OM (Herd et al. 2004; Løvendahl et al. 2018). For example, Herd et al. (2004) reported that variation in FE is largely explained by differences in feed intake (accounting for approximately 10–15% of variation), along with digestion and metabolic processes, indicating that FE is driven by animal-level physiological mechanisms. Similarly, Løvendahl et al. (2018) showed that selection for improved

FE can reduce enteric CH_4 intensity, mainly through reduced feed intake and associated changes in rumen fermentation, while lower feed intake may reduce the total quantity of manure and therefore total CH_4 emissions, it does not necessarily alter the composition or degradability of the excreted OM, which governs CH_4 production during manure storage. These findings suggest that the primary effects of FE on CH_4 production occur within the animal, which explains why no differences in manure CH_4 production were observed in the present study. For example, Nkrumah et al. (2006) reported that CH_4 production was 28% and 24% lower in low-RFI animals compared with high and medium-RFI animals, respectively. Importantly, these differences were observed in animals that consumed less feed while maintaining similar body weight and growth rates, indicating that the reduction in CH_4 production was primarily a result of lower feed intake rather than increased production. Similarly, Hegarty et al. (2007) observed that low RFI cattle produced approximately 25% less CH_4 per day compared with less efficient animals, largely due to lower feed intake and differences in digestion.

At the system level, manure contributes a smaller but variable proportion of total CH_4 emissions compared to enteric fermentation, with differences largely influenced by production system characteristics such as manure management and storage conditions. For instance, manure accounted for approximately 31% of total emissions in confinement systems but only 13% in pasture-based systems, reflecting the higher emissions associated with liquid manure storage compared to grazing-based systems (Dida et al. 2025). This supports the present findings, where dietary changes that typically influence enteric CH_4 did not result in measurable changes in manure CH_4 production.

Overall, these results indicate that manure CH_4 production is more strongly influenced by substrate characteristics and post-excretion conditions than by moderate dietary changes or FE differences (Sommer et al. 2007; Baral et al. 2018).

5.2 Total gas production

Total gas production was significantly higher in manure from feed-efficient cows compared to inefficient cows, particularly under the F55 diet (305 vs. 130

mL) and to a lesser extent under F70 (332 vs. 242 mL), while diet composition itself did not significantly affect TGP. Diet composition typically affects gas production through its influence on substrate availability, digestibility, and carbohydrate composition (Getachew et al. 2004; Amon et al. 2006). For example, Getachew et al. (2004) reported cumulative gas production values ranging from approximately 200–350 mL/g DM for highly fermentable substrates, compared with 150–250 mL/g DM for more fibrous feeds, indicating that substrate degradability strongly determines gas production. Similarly, Amon et al. (2006) demonstrated that gas production from stored slurry is strongly influenced by both the amount and degradability of OM, with higher dry matter content and greater availability of degradable substrates resulting in increased gas emissions during storage.

Dietary contrasts involving F:C ratio also affects fermentation intensity. Darabighane et al. (2024) reported that increasing concentrate proportion (F:C 0.35 vs. 0.65) resulted in significantly higher *in vitro* gas production and fermentation rate, indicating enhanced microbial activity with greater availability of fermentable substrates. However, the magnitude of this effect depends on how strongly diet composition alters substrate characteristics (Møller et al. 2014b; Miranda et al. 2016). In contrast, the present study showed no significant effect of diet composition (F55 vs. F70) on TGP, suggesting that the dietary contrast applied was not sufficient to modify the amount or degradability of OM in manure.

Feed-efficient animals are known to differ in digestion, metabolism, and nutrient partitioning, with studies reporting approximately 5–15% lower feed intake for similar production in more efficient animals (Herd et al. 2004) and genetic variation in FE of around 10–20% between animals (Berry & Crowley 2013). These differences in FE may influence the amount and composition of OM excreted (Huhtanen et al. 2021). Consequently, increases in TGP, which reflect overall microbial fermentation activity, do not necessarily translate into increased CH_4 production, as CH_4 formation depends specifically on H_2 availability and competing metabolic pathways (Bergman 1990; Janssen 2010).

5.3 pH and DM disappearance

The pH values (6.12–6.17) and DM disappearance (approximately 67.2–67.4%) were similar across all treatments, indicating stable fermentation conditions and comparable substrate degradation. These findings suggest that diet composition did not significantly influence the fermentation environment or extent of OM breakdown under the conditions of this study (Møller et al. 2014b; Miranda et al. 2016).

In manure systems, pH is primarily influenced by the balance between acid production and buffering capacity during microbial degradation of OM (Amon et al. 2006; De Vrieze et al. 2017). Amon et al. (2006) reported that slurry characteristics, including DM content and OM composition, strongly influence microbial activity and gas production, while pH remains relatively stable unless substantial changes in substrate composition occur. Similarly, De Vrieze et al. (2017) demonstrated that microbial community interactions during anaerobic digestion can stabilize intermediate products such as VFA, thereby maintaining relatively stable pH conditions despite variations in substrate input. This indicates that moderate dietary differences are unlikely to significantly affect manure pH unless they alter the quantity or degradability of OM entering storage, reinforcing that pH stability in manure systems is governed more by microbial regulation than by moderate variations in diet composition (Sommer et al. 2006).

Dry matter disappearance reflects the extent of substrate degradation and is closely related to the composition and degradability of OM (Amon et al. 2006; Makian et al. 2026). Diets rich in starch and sugars generally increase digestibility, whereas fibre-rich diets reduce degradation rates (Amon et al. 2006). Granja-Salcedo et al. (2016) reported that increasing concentrate inclusion improved OM digestibility, indicating enhanced microbial utilization of substrates. However, these effects depend on whether dietary changes significantly alter the composition and degradability of OM entering manure, which ultimately governs post-excretion fermentation processes (Møller et al. 2014b; Huhtanen et al. 2021).

In the present study, the narrow pH range further indicates stable microbial conditions. Since methanogenic activity is sensitive to pH, consistent pH values suggest that fermentation pathways were not altered across treatments (Janssen 2010; Dalby et al. 2021). Overall, the consistency in pH and DM disappearance indicates that both substrate availability and fermentation conditions were similar across treatments, limiting the potential for differences in CH_4 production (Dalby et al. 2021; Huhtanen et al. 2021).

5.4 Volatile fatty acids

The absence of differences in VFA profiles across treatments suggests that post-excretion fermentation pathways were not substantially altered by diet or FE. In manure systems, VFA represent intermediate products of anaerobic degradation and are influenced primarily by substrate composition and environmental conditions rather than dietary factors alone (Angelidaki & Ellegaard 2003; Baral et al. 2018; Dalby et al. 2021).

Studies on manure and anaerobic systems show that VFA are key intermediate products formed during the microbial degradation of OM, and their accumulation reflects the progression and balance of fermentation pathways rather than direct dietary effects (Baral et al. 2018). Similarly, Miranda et al. (2016), in a meta-analysis of CH_4 yields from dairy manure, highlighted that substrate characteristics and degradability are the main determinants of fermentation outcomes. Factors such as OM composition, VS content, and the proportion of readily fermentable versus structural carbohydrates influence the extent and rate of microbial degradation. These characteristics determine the production and distribution of intermediate products such as VFA, which reflect the progression of fermentation pathways. As a result, similar substrate inputs tend to produce comparable VFA profiles during manure fermentation.

In addition, Steed & Hashimoto (1994) demonstrated that CH_4 production from manure is strongly dependent on the biodegradability of OM, with fermentation intermediates such as VFA being rapidly utilized during methanogenesis. This rapid turnover of VFA may reduce the likelihood of detectable differences between

treatments, even when minor variations in substrate composition exist (Batstone et al. 2002; Appels et al. 2008).

During manure storage, microbial communities continuously degrade and convert organic substrates, which can reduce initial differences in substrate composition over time. For example, De Vrieze et al. (2017) reported that microbial succession and metabolic interactions during anaerobic digestion led to stabilization of intermediate products, resulting in relatively consistent fermentation patterns despite variation in initial substrate characteristics. The absence of differences in VFA profiles in the present study suggests that microbial interactions during manure fermentation buffered potential effects of diet composition, resulting in stable fermentation pathways across treatments. Page et al. (2014) reported that VFA concentrations in stored dairy manure initially increased due to active microbial fermentation, with acetate as the dominant acid, and subsequently decreased as these intermediates were further degraded during anaerobic digestion. This highlights that VFA are dynamic intermediates whose concentrations are governed primarily by microbial processes and fermentation stage rather than initial substrate characteristics.

Overall, the similar VFA proportions observed in this study suggest that substrate availability and environmental conditions were comparable across treatments, resulting in stable fermentation pathways. This indicates that VFA profiles in manure are influenced more by substrate characteristics and microbial activity than by moderate changes in diet or FE (De Vrieze et al. 2017; Dalby et al. 2021).

6. Conclusion

This study showed that FE and diet (F55 vs. F70) did not significantly affect CH_4 production from dairy manure. Although efficient cows produced more total gas, this did not lead to higher CH_4 production. Other parameters such as pH, DM disappearance, and VFA were also similar across all treatments. This indicates that manure exhibited similar behaviour during fermentation regardless of diet or efficiency group.

These findings highlight that mitigation strategies focusing only on animal diet may overlook an important source of emissions from manure. A combined approach that integrates both feeding strategies and improved manure management is likely necessary to achieve meaningful reductions in total CH_4 emissions from dairy systems. Additionally, the results emphasize the need for whole-system evaluations when developing sustainable livestock production practices.

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Popular Science Summary

Methane is a strong GHG that contributes to climate change. Dairy cows produce CH_4 during digestion, but CH_4 is also produced from manure when it is stored without oxygen. Most studies focus on CH_4 produced inside the cow, but CH_4 from manure is also important. Understanding how feeding affects CH_4 from manure can help reduce emissions from dairy farms.

This study looked at whether FE and diet affect CH_4 production from dairy cow manure. FE means how well a cow converts feed into milk. More efficient cows may produce manure with less undigested material. Two different diets with different forage and concentrate levels were also tested.

Manure samples were collected from dairy cows and tested in the laboratory. The samples were incubated, and CH_4 production, TGP, pH, DM disappearance, and VFA were measured. These measurements help show how manure breaks down and produces gas.

The results showed that CH_4 production from manure did not change with FE or diet. Efficient cows produced more total gas, but this did not increase CH_4 production. Other measurements were also very similar for all groups. This indicates that manure behaved in the same way regardless of diet or FE.

These findings suggest that changing diet alone may not reduce CH_4 emissions from manure. Improving FE also did not reduce CH_4 from manure. Therefore, better manure management, such as improved storage or biogas production, may be more effective for reducing CH_4 emissions.

Overall, reducing CH_4 emissions from dairy farms requires both good feeding strategies and improved manure management.

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Use of Writing Assistance Tools

I would like to acknowledge the use of digital tools that supported the preparation of this thesis. The writing assistance tool Grammarly was used to improve grammar, spelling, and clarity of the text. In addition, generative AI models were used to support language refinement, idea structuring, and general feedback on phrasing.

The use of these tools was carried out in accordance with the guidelines for the use of generative AI and digital support tools provided by the Swedish University of Agricultural Sciences (SLU). These tools were used solely to enhance the readability and presentation of the work. All scientific content, interpretations, and conclusions are my own, and I take full responsibility for the accuracy and integrity of the material presented in this thesis.

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