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Early-Life Respiratory Emissions of CO_2, CH_4, and N_2O in Pre-Weaned Dairy-Bred Calves

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ABSTRACT

Greenhouse gas (GHG) emissions from livestock are a major contributor to climate change, with cattle known to be the principal contributor through enteric fermentation, manure management, and metabolic processes. This study investigates the emission rates of CO_2, CH_4, and N_2O in pre-weaned calves aged 12 to 86 days, focusing on the transitional phase from a purely milk-based diet to the introduction of solid feed. Using a novel respiratory mask, repeated measurements of GHG emissions were collected from 65 calves to offer first insights into early-life respiratory GHG emissions from calves. All three gases increased with age, with CH_4 showing a much stronger age dependence than for CO_2 or N_2O, consistent with early developmental changes as calves begin to consume solid feed. Eructation events were observed only in calves older than 25 days, and were characterised by significant increases in CH_4, but not CO_2 or N_2O. Overall, CO_2 emissions dominated the GHG profile of the cattle, and although breath concentrations remained relatively stable, emission rates increased significantly with age, suggesting that changes in tidal volume and lung capacity were the primary drivers. Breed-specific differences were limited and sensitive to model structure; after adjusting for age using a generalised linear model, British Blue-cross calves exhibited higher CH_4 emissions and lower N_2O emissions compared to other breeds, though these differences were not statistically significant across all comparisons. These findings provide the first empirical characterisation of early-life respiratory greenhouse gas emissions in calves, offering baseline data to support improved modelling of livestock emissions and informing future mitigation strategies that target the pre-weaning period.

1 | Introduction

Greenhouse gas (GHG) emissions are a significant environmental concern, contributing to climate change and global warming. Ruminant livestock farming, while essential for global food security and economic stability, is a notable contributor to these emissions. The most recent estimates summarised by the Food and Agriculture Organisation (FAO) suggest that GHG emissions from livestock account for between 11.1% and 19.6% of total anthropogenic emissions (Gerber et al. 2013).

Among livestock, cattle and the production systems surrounding them are the largest sources of GHG emissions, with manure management, feed production, land use change, and energy use all significant factors (FAO 2017). However, enteric fermentation is the single largest source of emissions from cattle. Carbon dioxide (CO_2) is the most abundant compound emitted on the breath, but enteric fermentation produces significant amounts of methane (CH_4), a potent GHG (Hristov et al. 2013) — estimated to have a 27.2 times higher global warming potential than carbon dioxide (CO_2; global warming

potential over a 100-year time period,(IPCC 2021)). Additionally, emissions of nitrous oxide, although far smaller, further contribute to the GHG footprint due to their even greater global warming potential (Rivera and Chará 2021).

The enteric emissions profile of cattle is significantly influenced by their diet during the early life stages (Jami et al. 2013). Artificially reared dairy calves are typically separated from their dams within the first day of life and are then reared in pens with other calves of a similar age and fed milk replacer from buckets or an automatic calf feeder until weaning. In most systems, this takes place around 7–9 weeks of age. Solid feed is introduced to the calves early in life, but intake is normally low in the first few weeks and gradually increases as the animals grow and increases markedly when weaning from the milk takes place.

In the first few months, especially as calves transition from a milk-based diet to solid feed, the microbial composition of the rumen undergoes significant changes (Amin et al. 2021; Martinez-Fernandez et al. 2024). This transition is crucial as it activates rumen fermentation, a primary source of CH_4 emissions in adult cattle. Studies have indicated that calves introduced to solid feed earlier develop a more stable and efficient rumen microbial community, which can lead to lower CH_4 emissions in adulthood (Jami et al. 2013). Additionally, the early establishment of beneficial microbes can enhance feed efficiency and nutrient absorption, further mitigating enteric emissions (Zhuang et al. 2025). However, very few studies, if any, have investigated respiratory GHG emissions from calves, particularly as they transition from milk to concentrate feed.

In mature cattle, several methods have been employed to measure breath emissions, each with its own advantages and disadvantages. The GreenFeed system (GF), for example, is a widely used technique that captures and analyses breath emissions (McGinn et al. 2021). The cattle are attracted to the unit with bait feed, released under a hood and equipped with gas analysers. As the animals investigate the bait, their breath accumulates in the hood and is sampled and concentrations of CO_2 and CH_4 are measured. Electronic identification tags are automatically read to link the measurements to individual animals. The system provides several advantages, such as passive monitoring and rapidly gathering data from a large number of animals under field conditions, making it practical for real-world applications (Hammond et al. 2016; McGinn et al. 2021). However, measurements need to be collected over a period of weeks to months and it suffers from the disadvantage of breath being diluted with the ambient air, which can affect the accuracy of the measurements (Brunner et al. 2007; Hammond et al. 2016; Lassen et al. 2012). In particular, during grazing experiments, shifts in wind speed and direction can impact measurements (Ghassemi Nejad et al. 2024).

Respiration chambers provide another means to measure GHG emissions by isolating the animal and controlling environmental conditions (Storm et al. 2012). Consequently, the measurements tend to be highly accurate, but the approach can be labour-intensive and time-consuming and is not feasible for large-scale studies due to the limited number of animals that can be measured simultaneously. In addition, these chambers capture emissions from the entire animal, including enteric emissions, manure emissions, and emissions from the animal's skin. While this comprehensive capture can provide a thorough overview of total emissions, it presents a significant disadvantage when trying to isolate a specific emission source, such as enteric emissions. For example, Petersen et al. (2015) had to use rumen- and intestinal-cannulated cows in respiration chambers to specifically measure enteric N_2O emissions. This approach involves surgically inserting cannulas into the animals' digestive tracts, which is a significant intervention.

Despite these constraints, the use of both respiration chambers and the GF system has allowed the impact of dietary supplementation on GHG emissions to be assessed. One of the most promising strategies to reduce CH_4 emissions from cattle is the addition of 3-nitrooxypropanol (3-NOP) to their feed. 3-NOP acts by inhibiting methyl coenzyme M reductase, a key enzyme in the methanogenesis pathway, and has consistently demonstrated CH_4 reductions of up to 59% (Romero-Perez et al. (2015); Alemu et al. (2021); Meale et al. (2021)). Similarly, the addition of nitrates to feed have been shown to reduce CH_4 production by inhibiting the activity of methanogens in the rumen (Hulshof et al. 2012; Yang et al. 2016), with some studies showing reductions of up to 16% (van Zijderveld et al. 2011). This effect arises because nitrates act as an alternative hydrogen sink, diverting hydrogen away from methanogenesis and into other microbial pathways such as ammonia or N_2O production (Petersen et al. 2015).

While significant progress has been made in identifying dietary interventions and breeding practices (Jenkins et al. 2024) to reduce CH_4 emissions, important knowledge gaps persist. In particular, most N_2O research has focused on manure management rather than emissions from the breath (Knapp et al. 2014). Furthermore, little is known about GHG emissions from pre-weaned calves or how these emission rates change during the transition from a milk-based diet to solid feed. To our knowledge, no studies have directly quantified early-life respiratory CO_2, CH_4, and N_2O emission rates or examined their age-related development. This gap limits our ability to model lifetime emissions trajectories and identify when mitigation interventions may be most effective.

In this study, emission rates of CO_2, CH_4 and N_2O were measured from the breath of calves aged between 12 and 86 days old. Rather than using passive sampling which can be strongly influenced by changes in background concentrations of the target gases (Langford et al. 2022), here, emission rates are measured directly, using a respiratory mask. Sampling each animal at regular intervals, we present growth response curves for CO_2, CH_4 and N_2O, that capture the period when animals transition from a milk only diet through to consuming solid food in the form of barley straw and feed pellets. Additionally, within breed differences in emission rates are assessed.

2 | Methods

2.1 | Animals

A total of 65 cattle were included in the trial, comprising an even mix of males and females. All had Holstein dams (a dairy breed) and were born on a dairy farm, but were sired by bulls from three beef breeds—Hereford, Aberdeen Angus, and British Blue. Animals entered the study at various ages, between 12 and

45 days old and were sampled on up to six occasions over a four to 5-week period. The weight of each calf was measured at the start and end of the trial.

The calves were fed 4 litres of a milk replacer (Carr's Billington Vitality calf milk) at 8 am and 3 pm each day. In addition, they had ad lib access to barley straw and starter pellets (Davidson's rapid starter pellets, 2359). Breath measurements usually took place around four to 5 h after the first feed, although this varied depending on availability and the number of animals that required sampling on a given day.

Ethical approval for the study was granted by SRUC's Animal Experiments Committee (Approval number: AEX 2024-024 DAI).

2.2 | Breath Sampling

Measurements of CO_2 and N_2O were made using a MIRO multi gas analyser (MGA10-GP, MIRON Analytical, Wallisellen, Switzerland). The instrument uses direct laser absorption spectroscopy to provide precise concentrations of a range of gases with a time resolution of up to 10 Hz, but here was operated at 1 Hz. Although the MIRO can measure CH_4, a failed laser meant concentrations of this gas were instead measured using a separate RMT-200 Fast Methane Analyser (Los Gatos Research, CA, USA) which sampled at the same rate.

Breath samples were obtained directly from individual animals using a custom-built mask. The mask comprised a polycarbonate cylinder (15 cm diameter, 15 cm long) which was closed at one end. The closed end was connected to a respirator filter (3 M, DT-4001E-GF22) containing charcoal which helped to remove trace gases entering the mask. The mask entrance was covered with a silicone lid with 8 cm diameter hole which provided an effective seal around the animal's muzzle. Prior to each measurement, the mask opening was sealed using a silicone lid. This forced incoming air through the charcoal filter to provide a mask blank which was measured for 3 min. The mask was then placed on the animal and its breath sampled for a further 3 min. The emission rates of the three GHGs were calculated following (Li et al. 2022)

$$E_{exhaled} = (x_{breath} - x_{bg}) \cdot TV \cdot RR. \quad (1)$$

Where, ($E_{exhaled}$) is the emission rate in units of e.g. mg animal^{-1} h^{-1}, χ_{breath} is the median concentration of the GHG (mg m^{-3}) measured over the 3 min from the mask and χ_{bg} is the 3 min median concentration measured from the mask blank. The tidal volume (TV) was calculated based on the animal's body weight, assuming 8×10^{-6} m^3 per kg of body weight from (Dißmann et al. 2023). Each animal was weighed at the start and end of the study, and interim values were estimated based on a linear interpolation between the two weights. Respiration rates (RR) were counted for each animal from the high-resolution CO_2 data which clearly showed the respiration cycle.

Air was sampled from the mask at approximately 6 LPM through a ¼" (O.D.) stainless steel tube coated with Sulfinert. The 6-metre length of tubing was heated to maintain an internal temperature of 40°C, preventing condensation. The sampled air was then fed into a mobile laboratory housing the gas analysers.

Figure 1A shows the custom-built mask applied to a 2-week-old calf to measure the concentration and emission rate of the three GHGs from its breath and Fig. 1B highlights the background and breath phases of the measurement. In this instance, breath concentrations of CO_2 and CH_4 are significantly larger than the background, indicating emission. For N_2O, there was no difference between the breath and background samples, revealing that this animal was not producing N_2O from its breath at this time.

2.3 | Statistical Analysis

A statistical analysis of breath emission rates was undertaken to assess potential differences between calf breeds. Data were tested for normality using the Shapiro–Wilk test, and several transformations—including logarithmic, square root, and Box–Cox—were trialled. However, none achieved satisfactory normalisation. Consequently, a Generalised Linear Model (GLM) with a Gamma distribution and a log link function was selected for the analysis.

Breed and age were included as predictor variables in the GLM. Age was treated as a covariate to control for physiological development, which influences metabolic activity and greenhouse gas emissions. Although initial models included weight as a covariate, it was excluded from the final analysis due to methodological concerns. Specifically, weight was not measured at each breath sampling point but interpolated between start and end measurements. Moreover, weight was used to calculate tidal volume—a component of the emission rate calculation—introducing circularity and potential bias, making it unsuitable as an independent predictor.

To assess the impact of excluding weight, a sensitivity analysis was conducted and is presented in the Supplementary Information (Tables S8–S14). Including weight as a covariate improved statistical fit, but this may reflect mathematical coupling with the emission rate calculation rather than a true explanatory relationship. To avoid this potential confounding, the final models presented in Section 3.4 adjust only for age, providing a more robust basis for interpreting breed-level differences.

All statistical analyses were conducted in Python (Version 3.13) using the statsmodels, pandas, numpy, and scipy libraries. Breed was treated as a categorical variable, and model fitting was performed using iteratively reweighted least squares. Estimated marginal means (EMMs) and 95% confidence intervals were calculated for each breed at the mean age. Pairwise comparisons between breeds were conducted using linear contrasts with Bonferroni-adjusted p-values to control for multiple testing. Adjusted medians and interquartile ranges were derived from model predictions to facilitate comparison with raw emission distributions.

For CH_4, the analysis was restricted to respiratory emissions, with eructation events removed. Eructations, which result from microbial fermentation in the rumen, become the predominant source of CH_4 emissions as calves transition to solid feed and their digestive systems mature. However, including eructations in the analysis is challenging due to the nature of the sampling protocol. Breath samples were collected over a 3-min period, during which some animals produced multiple eructations while others produced none. Moreover, the volume of each eructation is unknown and cannot be reliably estimated from

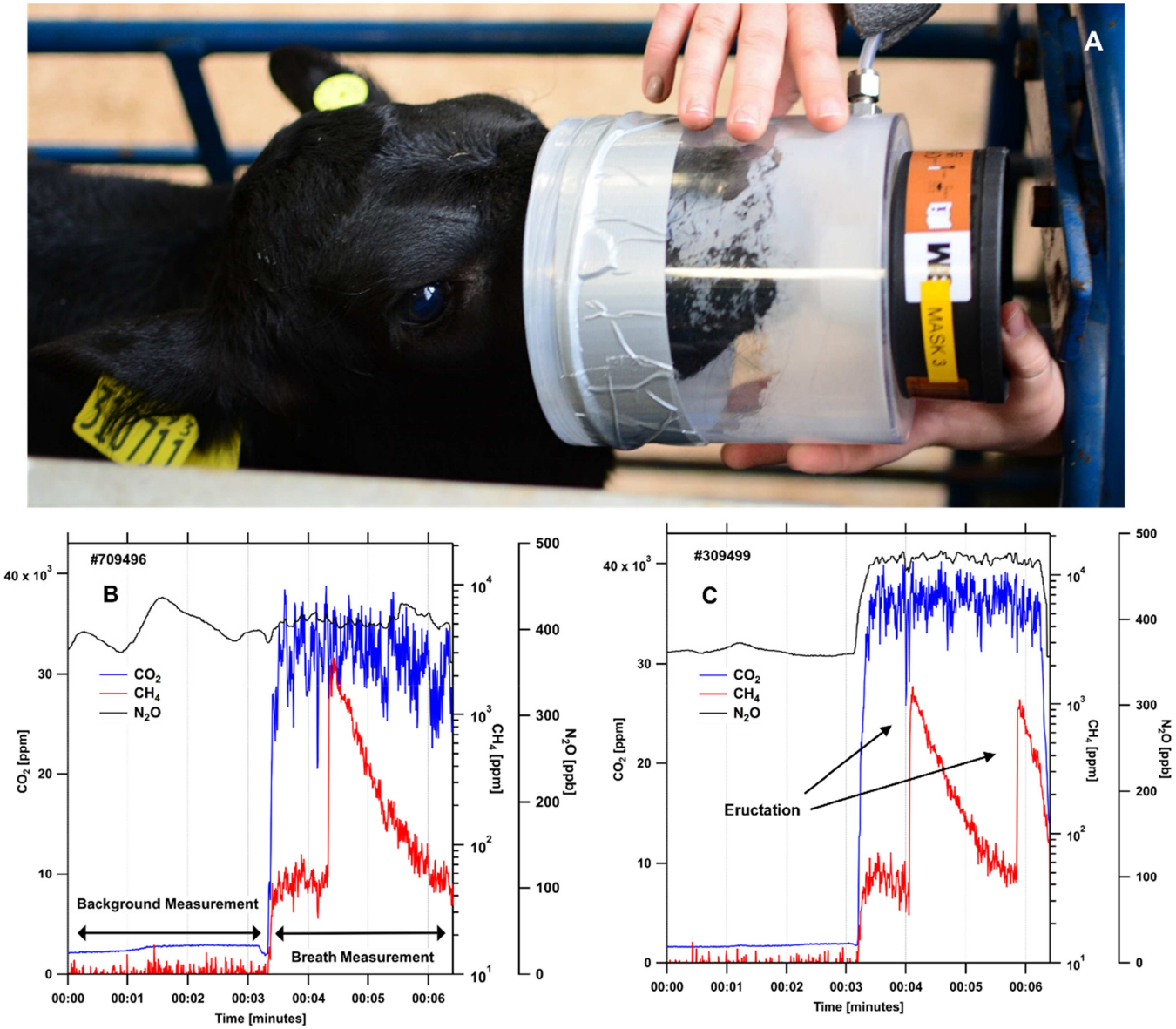

FIGURE 1 | A – Application of the breath sampling system to a 2 week old calf. B – Time series highlighting the time-line of sample capture, with 3 min of background (e.g. mask sealed and no calf present) and 3 min of breath sampling. B shows an example of an animal that didn't emit N_2O and C shows an animal that did emit N_2O.

the available data. This variability introduces substantial uncertainty and potential bias, making it difficult to compare eructation-derived emissions across individuals. For these reasons, eructations were excluded to ensure consistency and comparability in the analysis of respiratory CH_4 emissions.

3 | Results & Discussion

3.1 | Breath Sampling Summary

The cohort of cattle sampled using this experimental setup comprised 35 male and 30 females from three breeds: Hereford-cross ($n = 19$), Aberdeen Angus-cross ($n = 29$) and British Blue-cross ($n = 17$). In total, 383 breath samples were collected from 65 animals aged between 12 and 86 days old, with an average of six samples collected from each animal over a period of 4–5 weeks. British Blue-cross calves were on average 12 days younger than the Hereford-cross and Aberdeen Angus-cross ($p < 0.05$) (Table 1), but despite this difference there was no significant difference in average calf weight between breeds. Consistent with this, the estimated average daily weight gains (Interpolated weight/age) revealed British Blue-cross calves to have a higher ADG (2.2 kg/day) compared to the Hereford-cross (1.6 kg/day) or Aberdeen Angus-cross (1.8 kg/day). No differences in average respiration rates (34.7 breaths per minute) were observed between breeds.

3.2 | Breath Concentrations

Figure 2 shows histograms of breath concentrations (grey) and emission rates (blue) for CO_2 (A), N_2O (B), and CH_4 (C). Eructation events were observed in 15% of the collected samples and were characterised by large spikes in CH_4 concentrations. Figure 2C shows CH_4 breath concentrations with all eructation

TABLE 1 | Summary statistics for the cohort of 65 cattle used in the study.

	Hereford-cross	Aberdeen Angus-cross	British Blue-cross
Weight	69.42 ± 14.18	69.16 ± 13.52	67.6 ± 13.14
Age	41.82 ± 16.13$_a$	42.48 ± 15.5$_b$	**33.30 ± 10.77$_{a,b}$**
Respiration rate [BPM]	34.25 ± 5.8	35.05 ± 5.73	34.41 ± 6.0
N	102	181	99

Note: Bold text indicates significantly different means ($p < 0.05$). Subscripts denote the breed(s) to which the significant difference was found.

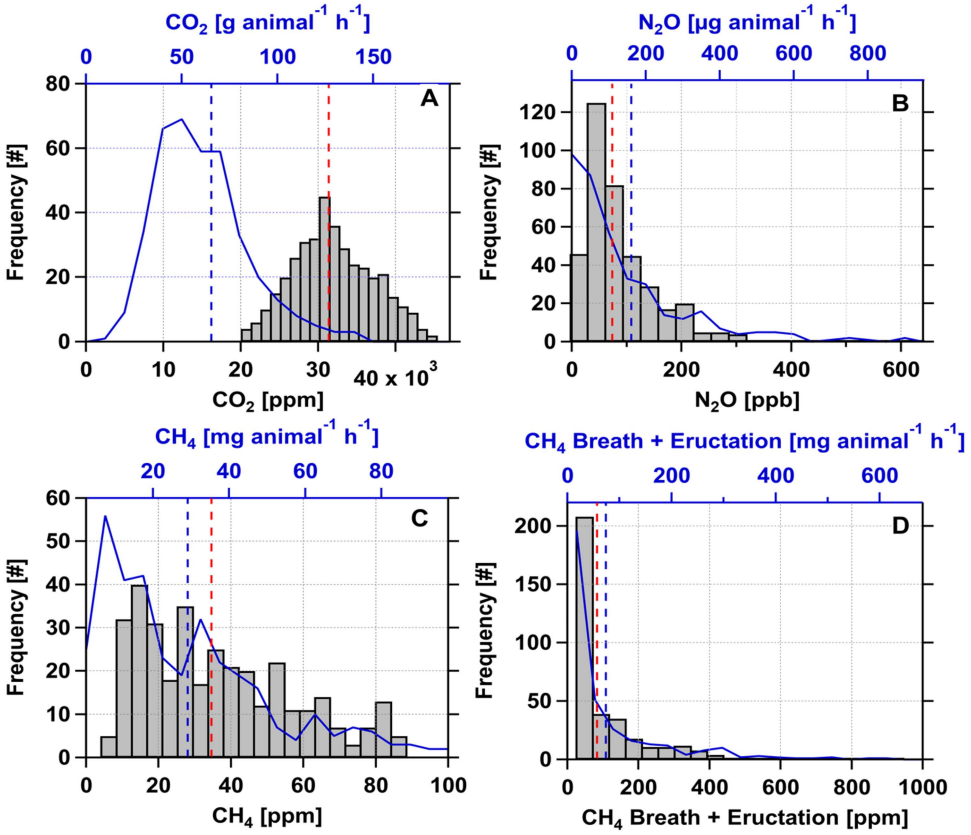

FIGURE 2 | Frequency distributions for both concentrations (Grey) and emission rates (blue) of breath CO_2 (A), N_2O (B), CH_4 (C) and CH_4 Breath + Eructations (D). Dashed red lines indicate concentration means and dashed blue lines show the mean emission rate.

events excluded and Figure 2D shows the same data with eructation events retained.

All concentration data represent the median of the 3-min breath sampling, minus the median of the 3-min background period. CO_2 concentrations (Figure 2A) were close to a normal distribution and ranged between 19,457 and 44,663 ppm with a mean average of 31,355 ppm. Concentrations of N_2O and CH_4 both showed a right-skewed distribution. For N_2O, concentrations ranged between 0 and 624 ppb with a median of 51 ppb. Concentrations were highly variable between animals and between samples from the same individuals.

Breath concentrations of CH_4 ranged between 2 and 84 ppm with a median of 30 ppm. Including periods of eructation (e.g. Figure 1B) increased the median concentration slightly to 35 ppm, but as shown in Figure 2D, peak CH_4 concentrations during eructation events could exceed 3,000 ppm. Eructation events were only observed from cattle aged > 25 days old. Animals had access to straw and feed pellets, which they likely began to consume around this time; this period generally corresponds to early rumen functional development, although rumen activity was not directly measured. With over a 100-fold increase in CH_4 during eructation events, even in young calves, these events appear to reflect emerging fermentation-related CH_4 release. No corresponding increases in either CO_2 or N_2O were observed in any of the 58 recorded eructation events, suggesting that these gases were not being produced at detectable quantities from the rumen at this early life stage.

3.3 | Breath Emission Rates

An analysis of the emission rates shows all three GHGs to have a right-skewed distribution. This reflects what is seen for N_2O and CH_4 concentration measurements, but for CO_2, the positive skew of the emission rates is likely linked to the growth rate of the cohort. Calves entered the study at different ages and were each sampled over a 4-to-5-week period. This allowed the relationship between both breath concentrations and emission rates to be assessed relative to calf age. Figure 3 shows the

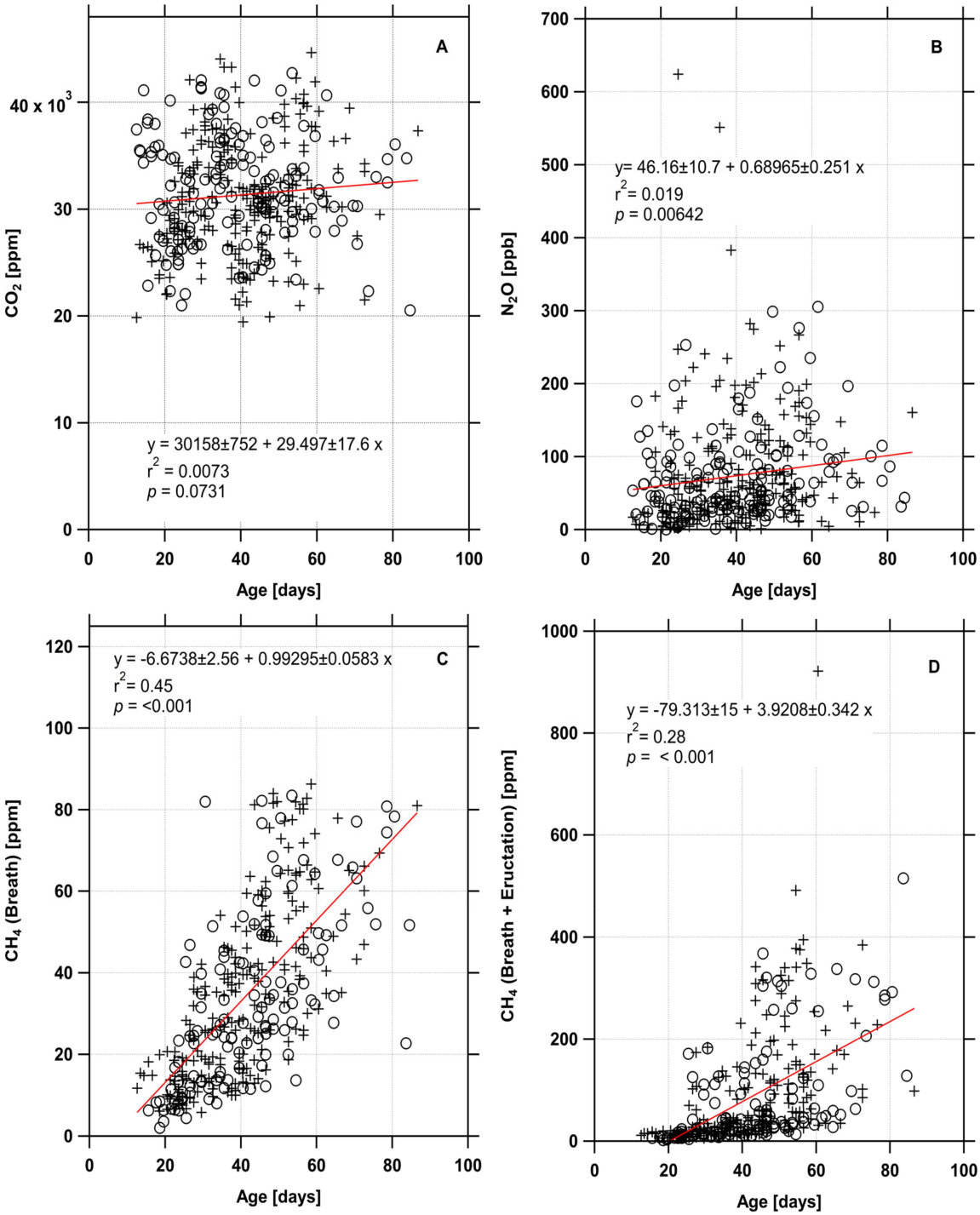

FIGURE 3 | Concentrations of CO_2 (A), N_2O (B), CH_4 (C) and CH_4 Breath + Eructations (D) measured in the breath of calves relative to their age. Crosses are males and circles are females.

results for concentrations and Figure 4 shows the emission rates. For breath concentrations, linear fits reveal increases for each GHG with respect to calf age. These fits were significant for N_2O ($p < 0.05$), CH_4 ($p < 0.001$) and CH_4 (Breath + Eructations) ($p < 0.001$). The fit for CO_2 was not significant ($p = 0.07$). However, the relationship between the emission rates of all GHGs, including CO_2, with calf age was significant ($p < 0.0001$). The fact that the emission rate increases significantly but not the concentration is indicative of an increased lung capacity and tidal volume, rather than a significant change in metabolic rate influencing the amount of CO_2 emitted on the breath.

3.3.1 | *Carbon Dioxide*

CO_2 emission rates ranged between 18 and 145 g animal^{-1} h^{-1}, with a median of 63.8 g animal h^{-1}. For an animal with the average cohort weight (69 kg), we can assume a tidal volume of around 0.55 L, similar to that of an adult human. Li et al. (2022) found humans to emit 25.5 and 29.9 g person h^{-1}. Respiration

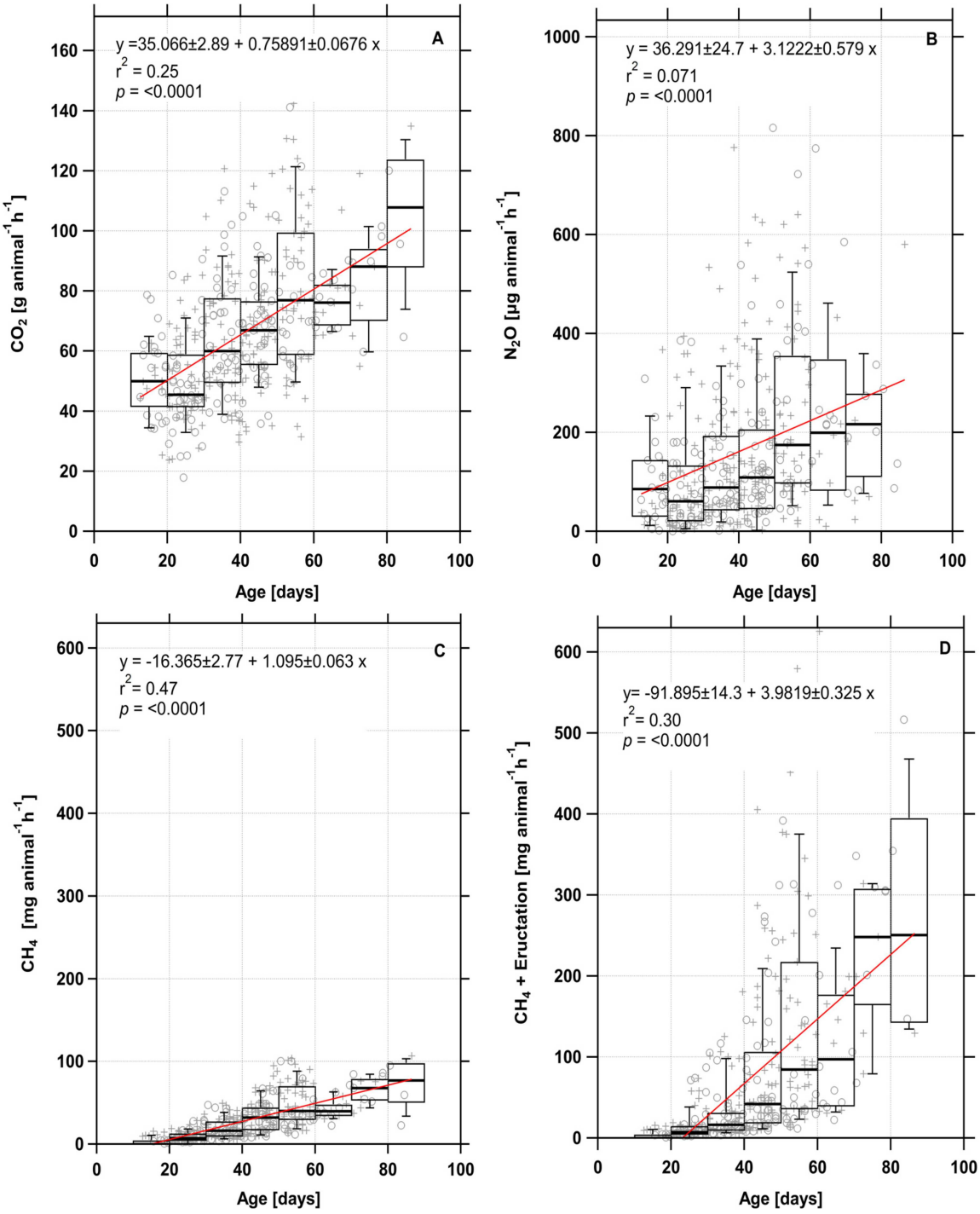

FIGURE 4 | Emission rates of CO_2 (A), N_2O (B) and CH_4 (breath only C, breath plus eructations D) measured relative to cattle age. Markers show values of indivdual measurements (Circles = female, crosses = male) and box and whisker plots are shown for the following age catagories: 10–20 days; 21–30 days; 31–40 days; 41–50 days; 51–60 days; 61–70 days; 71–80 days; 81–90 days. Whiskers show the 10th and 90th percentiles.

rates of calves are about double that of an adult human, and accounting for this difference yields very similar values.

Relatively few studies have presented breath emission rates of CO_2 from cattle, but there are some examples where daily emission rates have been calculated using emission monitoring systems centred around breath capture during feeding. For example Ryan et al. (2022) quantified emission rates of CO_2 and CH_4 from adult cattle using the GF system developed by Garnett et al (2012). CO_2 emissions ranged between 358 and 404 g animal^{-1} h^{-1} (Ryan et al. 2022). Comparing our measurements to those of adult animals involves extrapolation of our observations. Any extrapolation assumes the relationships found in Figures 3 and 4 remain linear, which may not be the case, especially considering the significant physiological changes that occur as the animals' transition to adulthood. Nonetheless, if values differ strongly, it serves to highlight where non-linearities might exist and direct future measurement efforts.

Here, we chose to extrapolate the GHG measurements to that of an adult animal, 500 days in age. We did so using two approaches: the first based on a straight extrapolation of the emission rate growth response curves, and the second, extrapolating the breath concentrations and calculating the emission rate based on known adult respiration rates (30 BPM (Dißmann et al. 2023)) and tidal volume (4 L^1). The second approach acknowledges some of the physiological differences that separate calves from adults and in particular the fact that the average daily weight gain observed during the trial, which ultimately dictated the lung tidal volume, may change significantly once the animals are fully weaned.

A direct extrapolation of the observed CO_2 emission rates from this study to that of an adult of 500 days yields a value of 414 g animal^{-1} h^{-1}, aligning closely with the range reported by Ryan et al. (2022). In contrast, an extrapolation based on the breath concentration response curve gives a value of 582 g animal^{-1} h^{-1}, ~35% above the reported range.

3.3.2 | *Methane*

In contrast to CO_2, significant differences are seen in the breath emission rates of CH_4 from non-eructating calves and humans. Emission rates ranged between 0.9 and 84 mg animal h^{-1}, with a median of 23 mg animal h^{-1}, with even the youngest animals (e.g. 12 days old) found to emit CH_4 on their breath. Li et al. (2022) found only one third of humans sampled to emit CH_4 on their breath, with an average of 4.03 mg person h^{-1}. More recently Dawson et al. (2023), found 31% of humans tested to emit CH_4 on their breath with a similar mean emission rate to that of Li et al. (2022) at 4.2 mg person h^{-1} [2]. Accounting for the reduced human respiration rate still results in a ~3 fold difference between calf and human CH_4 breath emission rates.

Methane emissions were considerably larger when eructation events were retained for the analysis. It is well documented that enteric emissions represent the single largest source of CH_4 emissions from adult cattle, accounting for 85%–90% of the total CH_4 emitted (Hindrichsen et al. 2005). From our analysis, it was clear that eructations containing elevated CH_4 were only observed in animals older than ~25 days, coinciding with the age at which calves typically begin consuming solid feed. Reliable quantification of the amount of CH_4 produced during these events is not possible as the volume and frequency of individual eructation's was unknown. Therefore, the absolute values presented here (Figure 4D) should be treated as indicative rather than fully quantitative.

The CH_4:CO_2 ratio is often used as an indicator of feed conversion efficiency, with lower ratios suggesting less energy lost as CH_4 and therefore more efficient conversion of feed into usable energy. In adult cows, this ratio varies with diet and intake, typically ranging from 0.01 to 0.22. Haque et al. reported an average ratio of 0.099 for adult Holstein cows reared on concentrate feed (Haque et al. 2014), while Lassen et al. (2012) found lower values for Holsteins (mean = 0.065) and Jerseys (mean = 0.05) fed on corn/grass silage, rapeseed meal, and soybean meal. Figure 6 shows a histogram of CH_4:CO_2 ratios calculated from both 3-min averaged breath concentrations and raw 1-second measurements. The average ratio for both datasets is similar (~ 0.0025), but the 1-second data capture individual eructation events, revealing transient increases up to 0.14. Interestingly, while CH_4 and CO_2 both increase during eructations in adults, this pattern is not observed in milk-fed calves. In calves, CH_4 is present during eructations, but CO_2 does not increase concurrently. This absence of CO_2 is consistent with limited ruminal fermentation activity at this stage, although rumen function was not measured directly.

The CH_4 may reflect early microbial colonisation or minor fermentation, but CO_2 production is likely too low to be detected or is being absorbed or metabolised differently. Therefore, while the low CH_4:CO_2 ratio in calves might superficially suggest high feed conversion efficiency, it more likely reflects limited rumen activity and should be interpreted with caution.

Reported CH_4 emission rates for adult cattle are significantly higher than those extrapolated from our emission and breath concentration growth response curves. Adult cattle are estimated to emit between approximately 8,000 and 18,000 mg animal^{-1} h^{-1}(Griffith et al. 2008). In contrast, extrapolating our CH_4 emission rates—excluding eructation events—yields a value of 531 mg animal^{-1} h^{-1}, while extrapolation based on breath concentrations (also excluding eructation events) results in a higher value of 2,313 mg animal^{-1} h^{-1}. These values are 8 to 15 times lower than reported values for adult cattle, which is expected given that emissions from enteric fermentation are not included. Essentially, our extrapolated values encompass CH_4 emissions from methanogens in the oral cavity and CH_4 dissolved in the blood and subsequently exhaled via respiration. While the production of some CH_4 is essential for the functioning of a healthy digestive system, our extrapolations provide a valuable perspective on the minimal emissions possible under idealised conditions. They serve as a theoretical benchmark against which practical mitigation efforts, such as dietary supplementation, can be measured.

3.3.3 | *Nitrous Oxide*

N_2O emissions were positively skewed with most samples falling between 0 and 150 μg animal h^{-1} with a median of 105 μg animal h^{-1}, and extreme values of 620 μg animal h^{-1}. Only three samples showed zero N_2O emissions, taken from three different individuals. Comparing these results to that observed by Dawson et al. (2023) reveals that calves emit less N_2O on their breath relative to humans. Dawson et al. (2023) found N_2O emissions to range between 261 and 283 μg person h^{-1} with a median of 273 μg person h^{-1}. Compared to the other GHGs, emission rates of N_2O were far more variable, with some animals emitting very little and other emitting up to six times the observed median.

Emissions rates of N_2O were very weakly correlated ($r^2 = 0.071$) with calf age, but the trend was significant ($p < 0.001$). Emission rates were small relative to previous studies of adult cattle which suggest values between 675 and 1,245 μg animal h^{-1} (Parker et al. 2018). Most recently, Petersen et al. (2015) reported average emission rates of 2,700 μg animal h^{-1}, with values dependent on NO_3^- intake. Median emission rates during this study were just 105 μg animal h^{-1}. Accounting for the apparent increase with calf age we can extrapolate the emission rate to that of an adult by assuming an age of 500 days. This yields an emission rate of 1,600 μg animal h^{-1}, which is within the range of previous measurements. Extrapolation via the

concentrations gave a value of 5,067 ug animal^{-1} h^{-1}. As with CH_4, the concentration extrapolation yields significantly larger values. This reflects the likely non-linearities that are expected in the growth response curves which could not be captured within the age range of our cohort. Breath concentrations, particularly for N_2O and CH_4 may relate strongly to the rapidly developing microbial community in the calf's oral cavity and digestive system. Previous work has shown that these microbial communities are likely to change and stabilise over time, particularly as the animals are fully weaned. For CO_2, primarily controlled by metabolic rate rather than microbial community, differences in breath emission rate- and breath concentration extrapolations was minimal.

The introduction of dietary nitrate as a CH_4 mitigation strategy in dairy cows has been found to inadvertently increase N_2O emissions. When cattle consume nitrate-rich feed, microbial activity in the rumen converts nitrate (NO_3^-) to nitrite (NO_2^-). Petersen et al. (2015) observed a transient accumulation of nitrite in the rumen fluid shortly after feeding, which suggests that the enzymes involved in dissimilatory nitrate reduction are induced in a cascade-like manner by the respective substrates. Some of the nitrite is absorbed through the rumen wall into the bloodstream and can be excreted in saliva. This leads to contact between dissolved nitrate and degradable organic matter in the oral cavity, where there is potential for interaction with denitrifying organisms (Smith et al. 1999; Granli et al. 1989). Petersen et al. (2015) postulated that N_2O emissions were therefore not enteric, but rather, originated from the oral cavity. A theory that was further supported by the lack of correlation between CH_4 and N_2O emissions direct from the rumen. Our measurements add credence to this theory, with N_2O emission rates remaining unchanged during periods of eructation. In addition, we found a weak, but significant ($p < 0.001$) correlation between the N_2O emission rate and the time since last feed (Figure 5).

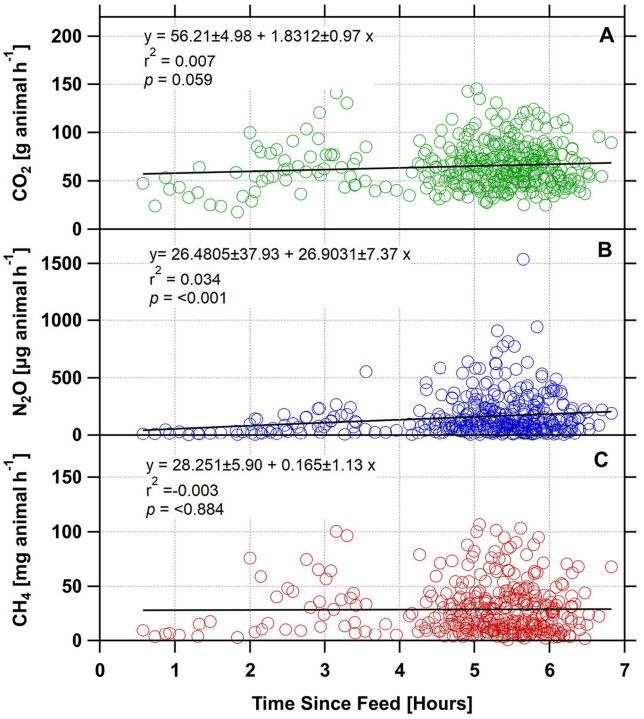

FIGURE 5 | Emission rates of CO_2 (A), N_2O (B) and CH_4 (minus eructations) (C) in relation to time since last feed.

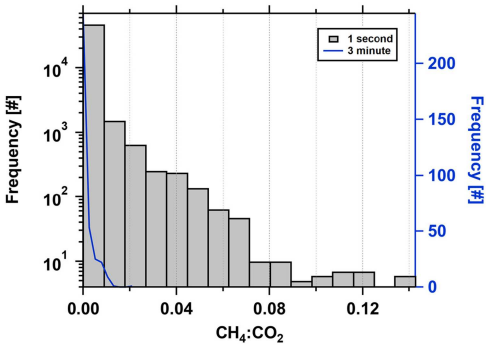

FIGURE 6 | Histogram of CH_4:CO_2 concentration ratios measured from cow breath. Grey bars represent 1 s data, blue line represents the 3 min average breath sample.

3.4 | Statistical Analysis of Emission Rates

Median emission rates for CO_2, CH_4 and N_2O are summarised in Table 2 for each breed, together with the adjusted means derived from the GLM analysis after accounting for the effect of age. Detailed results of the GLM for each gas are shown in Tables S1–S7 of the Supplementary Information.

The GLM analysis indicated that breed did not have a statistically significant effect on CO_2 emissions after adjusting for age. British Blue-cross calves exhibited slightly higher adjusted mean emissions (66.42 g animal^{-1} h^{-1}) compared to Aberdeen Angus-cross calves (62.94 g animal^{-1} h^{-1}), but this difference was not statistically significant (coefficient = 0.0539, $p = 0.171$; adjusted $p = 0.512$). Similarly, no significant difference was observed between Aberdeen Angus-cross and Hereford-cross calves (coefficient = 0.032, $p = 0.393$; adjusted $p = 1.000$). Age had a significant positive effect on CO_2 emissions (coefficient = 0.0118, $p < 0.001$), indicating that older calves emitted more carbon dioxide. This association is consistent with increased tidal volume and lung capacity linked to physiological development, rather than a direct increase in metabolic rate.

For CH_4 respiratory emissions (contributions from eructations excluded), British Blue-cross calves exhibited significantly higher emissions compared to Hereford-cross calves. The adjusted mean CH_4 emission for British Blue-cross calves was 28.55 mg animal^{-1} h^{-1}, while Hereford-cross calves had an adjusted mean of 21.10 mg animal^{-1} h^{-1}. The coefficient for British Blue-cross in the GLM was 0.3022 ($p = 0.0056$), indicating a statistically significant difference that remained significant after adjustment for multiple comparisons. In contrast, the difference between British Blue-cross and Aberdeen Angus-cross calves (24.07 mg animal^{-1} h^{-1}) was not statistically significant (adjusted $p = 0.0958$), nor was the difference between Aberdeen Angus-cross and Hereford-cross calves (adjusted $p = 0.3301$).

As for CO_2, and despite eructation events being excluded from the analysis, age remained a strong predictor of CH_4 emissions (coefficient = 0.0461, $p < 0.001$), indicating that CH_4 production increases with physiological maturity. This likely reflects the early colonisation of methanogenic microbes in the respiratory

TABLE 2 | Median and interquartile range of green House gas emissions from Aberdeen Angus-cross, British Blue-cross and Hereford-cross calves. Adjusted means are those calculated using a GLM model to account for age related variation.

	Breed	Median | IQR |	Adjusted mean ± SE
CO_2 [g animal^{-1} h^{-1}]	Aberdeen Angus-cross	63.61 | 46.14–79.07|	62.94 ± 0.02
	British Blue-cross	57.79 | 43.98–72.52|	66.42 ± 0.03
	Hereford-cross	64.90 | 54.04–78.46|	65.00 ± 0.03
CH_4 [mg animal^{-1} h^{-1}]	Aberdeen Angus-cross	23.56 | 7.90–38.27|	24.07 ± 0.05
	British Blue-cross	16.64 | 6.45–33.44|	**28.55 ± 0.06**
	Hereford-cross	26.82 | 12.29–40.88|	21.10 ± 0.07
N_2O [μg animal^{-1} h^{-1}]	Aberdeen Angus-cross	118.34 | 58.22–242.11|	173.44 ± 0.08
	British Blue-cross	81.23 | 40.30–152.20|	118.87 ± 0.11
	Hereford-cross	116.13 | 55.90–227.30|	153.64 ± 0.11

Note: Aberdeen Angus-cross was the baseline breed to which the others are compared. Bold values indicate statistically significant differences based on a *p*-values using the Bonferroni correction from the GLM analysis.

and upper digestive tract as animals begin consuming solid feed, rather than ruminal fermentation alone.

For N_2O emissions, British Blue-cross calves exhibited significantly lower emissions than Aberdeen Angus-cross calves. The adjusted mean N_2O emission for British Blue-cross calves was 118.87 μg animal^{-1} h^{-1}, whereas Aberdeen Angus-cross calves had an adjusted mean of 173.44 μg animal^{-1} h^{-1}. The coefficient for British Blue-cross in the GLM was −0.3778 ($p = 0.006$), indicating a statistically significant reduction in emissions. However, the difference between British Blue-cross and Hereford-cross calves (153.64 μg animal^{-1} h^{-1}) was not statistically significant (adjusted $p = 0.2873$), nor was the difference between Aberdeen Angus-cross and Hereford-cross calves (adjusted $p = 1.000$). Age was positively associated with N_2O emissions (coefficient = 0.0187, $p < 0.001$), though the relationship was weaker than for CH_4 and more comparable to CO_2.

To assess the robustness of these findings, a sensitivity analysis was conducted using models that included weight in addition to age as covariates. These models showed improved statistical fit and revealed changes in statistical significance for some breed-level comparisons (Tables S8–S14). For example, the difference in N_2O emissions between British Blue-cross and Hereford-cross calves became statistically significant in the weight-adjusted model (adjusted $p = 0.0098$), though it was not significant in the age-only model (adjusted $p = 0.2873$). In contrast, no breed-level comparisons for CH_4 emissions were statistically significant in the weight-adjusted model, including the comparison between British Blue-cross and Aberdeen Angus-cross calves (adjusted $p = 1.000$). Similarly, no breed-level comparisons for CO_2 emissions were statistically significant in either model.

These modelling sensitivities underscore the broader challenge of interpreting breed-related differences in emissions during early life. The pre-weaning phase represents a period of rapid, often exponential growth, during which physiological and metabolic processes change quickly. Emission rates are likely to vary substantially over short timescales, and accurately accounting for these changes is challenging, particularly when measurements are limited to brief sampling windows, or interpolated metrics (e.g. weight). While breed-level trends were observed, these may be influenced by transient developmental factors rather than stable genetic differences. For example, it was notable that statistical differences were observed only for the British-Blue calves, who also showed a much larger ADG compared to the other breeds. As such, the results presented here should be viewed as indicative of early-life emission dynamics rather than definitive breed comparisons.

4 | Conclusions

This study provides detailed insights into greenhouse gas emissions from pre-weaned calves, focusing on CO_2, CH_4, and N_2O during the transitional phase from a milk-based diet to solid feed. Measurements using a custom respiratory mask revealed significant age-related variation in emission rates, with breed-level differences evident in some cases but generally not statistically significant across all comparisons. British Blue-cross calves exhibited higher age adjusted CH_4 emissions and lower N_2O emissions relative to other breeds, though these patterns were sensitive to model structure and covariate inclusion.

CO_2 emissions dominated the overall greenhouse gas (GHG) profile during early development, reflecting high metabolic rates and increasing tidal volume rather than changes in breath concentration. CH_4 emissions increased notably after 25 days of age, coinciding with the age at which calves typically begin to consume solid feed, a period generally associated with early rumen functional development. Eructation events, observed only after this dietary transition, contributed to large spikes in CH_4 concentrations, suggesting the emergence of fermentation-related CH_4 release, although specific sources were not directly quantified. Interestingly, no corresponding increase in CO_2 was observed during these events, in contrast to patterns seen in adults. N_2O emissions were highly variable and relatively minor, with temporal patterns consistent with a contribution from oral microbial processes, though this was not directly measured. Like CO_2, N_2O concentrations did not increase during eructations.

Extrapolation of CO_2 emission rates to adult cattle yielded values consistent with published data, while extrapolation based on breath concentrations overestimated emissions, highlighting the non-linear physiological changes that occur during growth. These findings underscore the complexity of interpreting GHG emissions during early life and suggest that short-term measurements may not reliably predict long-term emission profiles.

Future research should extend these observations into the post-weaning period, when stabilisation of the rumen microbiota, dietary transitions, and ongoing physiological development are likely to influence greenhouse gas emission dynamics. This should include a targeted measurement strategy capable of quantifying both the rates of emissions during eructation events and their frequency of occurrence. A better understanding of these processes will support efforts to integrate environmental sustainability with livestock productivity.

Author Contributions

Ben Langford: conceptualisation, funding acquisition, methodology, investigation, formal analysis, visualisation, writing – original draft, writing – review and editing. **Johanna M. C. Brans:** investigation, writing – review and editing. **Claire Broadbent:** investigation, writing – review and editing. **Marie Haskell:** conceptualisation, funding acquisition, methodology, writing – original draft, writing – review and editing. **Laura Nicoll:** investigation, writing – review andediting. **Neil J. Mullinger:** methodology, writing – review and editing. **Carol-Anne Duthie:** conceptualisation, funding acquisition, methodology, writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The dataset supporting this study is freely available from the Zenodo data repository at https://doi.org/10.5281/zenodo.17466093.

Endnotes

[1]Based on 8 ml per kg and an assumed adult weight of 500 kg.

[2]Figure calculated from Dawson et al using the average CH_4 concentration for CH_4 producers (15 ppm at 37°C and 101.3 kPa), a respiration rate of 16 breaths per minute and a tidal lung volume of 500 ml.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
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