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Enteric methane concentrations from Boran steers determined with a laser methane detector under open-pen conditions and in the respiration chamber

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The Laser Methane Detector (LMD) has been proposed as a means of measuring methane (CH_4) emissions from ruminants in their natural rearing environment and ranking different diets and individual animals based on their CH_4 emission potential. The present study aimed to test the use of the LMD for ranking diets, animals, and periods of the day in a typical sub-Saharan African setting. The study involved 18 Boran steers, fed three different forages (Brachiaria, Napier, and Rhodes grass), with CH_4 emissions measured on the first day with the gold standard respiration chamber and on the subsequent day with the LMD in an open pen during 22 hours per day. Following a series of data correction steps, the mean LMD CH_4 concentrations were found to be lower than the mean chamber CH_4 concentrations, with a low overall correlation between the two (r_s=0.29). Neither chamber CH_4 nor LMD CH_4 were influenced by forage type. Both approaches recorded highest mean CH_4 concentrations directly after feeding and lowest at night. Although the results demonstrate the ability of the LMD to capture diurnal CH_4 emission patterns and forage-related differences in CH_4 emissions, the ranking of individual animals' CH_4 emission deviated from the chamber rankings. This was partly due to different feeding schedules between the chamber and the open pen, but also to abiotic environmental factors that affected the LMD readings and could not be fully removed through systematic data cleaning procedures.

KEYWORDS
diurnal emission patterns, enteric methane, field conditions, forage diets, tropical environments

1 Introduction

In the context of climate change, livestock production is under a lot of pressure as one of the agricultural sectors contributing substantially to greenhouse gas (GHG) emissions (Reisinger et al., 2021). Especially ruminant systems are in the focus of critique and subject to (national) GHG inventories (Dittmer et al., 2023), as enteric methane (CH_4) emissions of domesticated large and small ruminants account for 80% of the annual agricultural CH_4 release globally (Reisinger et al., 2021). To render ruminant systems more sustainable, multiple options for emission reduction have been identified and are being explored in detail. Next to breeding strategies (Denninger et al., 2020) and improvements in animal health (FAO, 2023), emission reductions through feeding and grazing management are of specific interest (Sorg, 2022). In particular, the use of natural or synthetic substances with potential to reduce enteric CH_4 production through interaction with feed components or the microbial fermentation and methanogenic processes in the rumen receives substantial scientific attention (Ungerfeld et al., 2022). To quantify the effectiveness of the suggested interventions, it is important to measure enteric CH_4 emissions before, during, and after the intervention, or of a control group *versus* treatment groups. Up to date, animal respiration chambers, undisputedly regarded as the gold standard for measuring enteric CH_4 emissions from ruminant livestock, are associated with high installation and running costs, limiting their widespread application, particularly in resource-constrained regions (Denninger et al., 2020; Zhao et al., 2020; Sorg, 2022). Further obstacles are a modified measurement environment, a low number of animals that can be measured per study, animal welfare considerations, a high labour demand, and the considerable expertise required in running the units and associated instrumentation (Chagunda, 2013; Denninger et al., 2020; Zhao et al., 2020; Sorg, 2022). Consequently, other approaches such as the GreenFeed® system (C-Lock Inc., Rapid City, SD, USA) have gained popularity, with an increasing number of countries in sub-Saharan Africa currently installing such units. GreenFeed offers distinct advantages over the classical respiration chamber as it is mobile, can be installed indoors or outdoors, and newer units are also capable of accommodating both large and small ruminants (Thompson and Rowntree, 2020; Zhao et al., 2020; Tedeschi et al., 2022). However, similar to respiration chambers, the system has some limitations. Methane measurements are only taken when an animal is feeding at the station, precluding the capture of the (reduced) exhale emissions when the animal is at rest (Roessler et al., 2018). The device also requires regular calibration and maintenance, incurs comparatively high purchase and operational costs, and its use is dependent on continuous internet connectivity for real-time data transmission to the provider (Brennan et al., 2024).

As a consequence, other techniques such as the Laser Methane Detector (LMD), originally designed to detect leaks in gas pipelines, have been employed in ruminant livestock for about 15 years (Chagunda et al., 2009; Sorg, 2022). The LMD provides notable advantages, particularly in terms of its rather low costs (about 10% of the costs of a GreenFeed system), ease of portability within and across country borders, self-calibration, and direct use in farmers' herds (Chagunda et al., 2013; Ricci et al., 2014; Kobayashi et al., 2021a; Bekele et al., 2022). While the initial tests from 2009 onwards were done with the first-generation LMD model offered by Tokyo Gas Engineering Solutions (Chagunda et al., 2009, 2013), the second-generation devices, namely the LaserMethane-mini (LMm) and LaserMethane-mini green (LMm-G) were increasingly in use since 2015 (Sorg, 2022). Today, the new LaserMethane Smart model is available, and the older models are no longer supported with Gas Viewer software updates by the company (T. Yokota, personal communication, January 2023).

Irrespective of the specific model, LMD devices provide CH_4 column density (ppm-m) readings between the operator and the target animal, with column density representing the integrated concentration of a gas across a measured path length. By measuring the distance to the animal's nostrils and dividing the column density by this value, the CH_4 concentration in exhaled air is obtained in ppm (Chagunda et al., 2009; Sorg, 2022). Thus, the device enables measurements of exhaled CH_4 concentration within the animals' actual rearing environment, be it barn or pasture, with data recorded on the operator's portable device, i.e., Android tablet or smartphone (older versions), on-board data storage (newer releases), and storage on a cloud server (latest model; https://www.crowcon.com/de/products/portables/laser-methane-smart/#specs). In the latter case, continuous internet connection may again be an issue in rural areas with poor communication infrastructure.

A critical challenge to the use of the LMD in the early days of its application in animal science was the lack of a standardized measurement protocol. This gap has been systematically addressed over the past 15 years. Today, it is common that (i) the operator should preferably point the laser beam in an angle of 90° and from a distance between 1 and 3 m to the animals' mouth/nostril area, (ii) a measurement period should last from a minimum of 2 to a maximum of 10 minutes, with a recording interval of 0.1 - 0.5 seconds, and (iii) at least three consecutive measurement days per animal should be implemented (Chagunda et al., 2009; Doran, 2015; Sorg et al., 2018b; Boré et al., 2022; Sorg, 2022). Outlier data points, which are often characterized by extremely high CH_4 densities, are frequently discarded based on visual inspection of plotted profiles or in relation to the mean value of a given profile (Niero et al., 2020; Kobayashi et al., 2021b; Sorg, 2022). The data set is then separated into two normally distributed functions with high and low CH_4 concentration, which represent eructation and respiration events (Ricci et al., 2014; Kobayashi et al., 2021b; Sorg, 2022). Furthermore, several studies ignore the "troughs", i.e., the regular individual readings of low CH_4 concentration that represent air inhale, while the exhale recordings with CH_4 mini peaks (normal exhale) and major CH_4 peaks (eructation, regurgitation) are retained (Chagunda et al., 2009; Sorg et al., 2018a; Niero et al., 2020). Currently available LMD studies commonly correct for background CH_4 concentrations at the study site, especially when measuring in barns with many animals, either by using the offset function of the instrument (Chagunda et al., 2013; Mapfumo et al., 2018) or by subtracting the minimum CH_4 concentration of a measurement period or profile as the background value (Doran, 2015; Pinto et al., 2020).

Since the laser beam has to be pointed to the mouth/nostril area, the measured animal often needs to be restrained (Doran, 2015), which may impact the exhale CH_4 concentration (Roessler and Schlecht, 2021). Therefore, parallel and time-synchronized voice recording on placement of the laser beam on or off the mouth/nostril area is required for data cleaning (Roessler and Schlecht, 2021).

Although numerous scientists used LMD devices for ruminant studies over the past 15 years, only one dissertation investigated its application for capturing diurnal CH_4 emission profiles in sheep through three daily measurements, using respiration chambers as the reference method (Doran, 2015). The limited evidence calls for expanding diurnal LMD measurements under outdoor conditions, particularly in tropical regions, where only five LMD studies have been published to date (Grobler et al., 2014; Mapfumo et al., 2018; Pinto et al., 2020; Kobayashi et al., 2021b; Bekele et al., 2025). Given the financial or logistical constraints that limit access to respiration chambers or GreenFeed systems in many tropical research institutions, the LMD, despite its mentioned limitations, still offers a valuable alternative (S. Velayudhan, personal communication, September 2024). Accordingly, our study used respiration chamber measurements as a reference method to evaluate the use of the LMD to (1) capture diurnal CH_4 concentration profiles and to (2) rank exhale-air CH_4 concentrations for time periods within a day, including (2a) different tropical forages, and (2b) individual tropical cattle to assess the device's potential for outdoor CH_4 emission monitoring in tropical environments.

2 Materials and methods

2.1 Animals and feeding

The study was conducted at the Mazingira Centre, International Livestock Research Institute (ILRI), Nairobi, Kenya, from June to July 2018 (animal ethics commission approval no: IACUC-RC2017-15). Eighteen Boran steers with a mean live weight of 216 ± 5.8 kg and an age of 16 to 20 months were used. The steers were allocated to three forage groups (FG) offered Napier grass (*Pennisetum purpureum* Schumach var. Kakamega 1), Rhodes grass (*Chloris gayana* Kunth var. Boma) and so-called Brachiaria grass (*Urochloa brizantha* (Hochst. ex A.Rich.) R.Webster) with only one grass fed to each group throughout the study. The chemical composition of grasses is shown in Table 1. Grass was harvested daily, chaffed to 2–4 cm particle size and then offered fresh to the animals at 110% level of their previous day's intake. Pre–weighed feed was offered as a single portion in the morning in the respiration chamber to avoid any disturbance of the methane–measurement cycle. In contrast, in the open–air pens where the LMD measurements took place, the daily feed allowance was divided into three equal portions offered at 09:00, 12:00, and 16:00 to prevent overfilling of the smaller feeding troughs that could not hold the full daily ration. Each animal had free access to drinking water and a mineral block at all times. Feed offered and refused was quantified daily for each individual animal to calculate feed and nutrient intake. Representative samples of feed offered and refused were collected every day, dried, and analysed for the concentration of dry and organic matter, crude protein, and acid detergent fiber according to standard protocols as outlined by Korir et al. (2023). Animals were weighed twice a week before morning feeding using a digital livestock scale (Gallagher Australia, Weigh Scale W210, accuracy 0.5 kg). All animals were well acquainted with the experimental handling procedures and could be approached to less than one meter distance without showing signs of fear.

While feeding the experimental rations started in the first week of May, the chamber and LMD measurements for this study took place during three experimental measurement phases that lasted from 5 to 11 June, 26 June to 7 July, and 10 to 16 July. During each of the three measurement phases, six steers, two per FG, were measured. In each phase, one animal per FG entered the respiration chamber on Day 1 and was measured with the LMD in the open pen on Day 2. On that same Day 2, the second animal of each FG was placed in the chamber and subsequently measured with the LMD on Day 3, when the first animal of each FG returned to the chamber for its second time. This alternating schedule proceeded until each of the six animals measured in a particular phase had completed three respiration chamber measurements and three LMD measurements, marking the end of the measurement phase. In the second and third measurement phase, two different animals per FG were measured in the same way (Supplementary Resource S1).

2.2 Respiration chamber measurement

Three open-circuit respiration chambers (No Pollution Industries, Edinburgh, UK) designed for cattle were used. The operation principle of these chambers has been described by Goopy et al. (2020). Briefly, each steer was housed individually in an airtight respiration chamber for approximately 22.5 hours, from 10:00 a.m. to 08:30 a.m. the following day. The chambers were located within a single large room. A ceiling light positioned between the chambers and the adjacent computer room remained switched on day and night, providing a uniform and moderate

TABLE 1 Mean (± standard error of the mean, SEM) dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fiber (ADF), and gross energy (GE) contents of Napier (*Pennisetum purpureum* var. Kakamega 1), Rhodes (*Chloris gayana* var. Boma) and Brachiaria (*Urochloa brizantha* var. Xaeres) grasses.

Grass	DM (g/kg FM)	OM (g/kg DM)	CP (g/kg DM)	ADF (g/kg DM)	GE (MJ/kg DM)
Napier	142 ± 8.9	856 ± 5.8	96 ± 3.3	405 ± 6.5	16.3 ± 0.08
Rhodes	233 ± 6.2	898 ± 4.9	80 ± 3.5	442 ± 5.0	17.2 ± 0.09
Brachiaria	206 ± 4.1	887 ± 3.0	83 ± 2.7	396 ± 7.2	16.8 ± 0.06

FM, fresh matter; for neutral detergent fiber (NDF) content see Korir et al. (2022).

illumination across all chambers. Following animal removal, the chamber was cleaned and the feed trough replenished between 08:30 a.m. and 10:00 a.m. in preparation for the next measurement cycle. Methane concentrations, expressed in parts per million (ppm), were continuously measured at both the inlet and outlet of the chamber airflow at 10-minute intervals using a cavity ringdown laser absorption spectrometer (Picarro G2508 analyzer). The chamber environment was maintained at a temperature of 22°C, relative humidity at 50 %, and a constant airflow rate of 80 L per second.

2.3 LMD measurement

Throughout the three measurement phases, a single LMD device equipped with a set of spare rechargeable batteries was operated by one single person (first author), who had received intensive training in the use of the device beforehand. For each animal, the LMD measurements began once it had been moved from the respiration chamber back to its individual pen (1.90 m × 2.87 m). Measurements were conducted between 10:00 a.m. and 07:00 a.m. the following day in a naturally ventilated barn, the roof of which was fitted with a sailcloth. During the night, three light bulbs that were evenly distributed across the pens, provided moderate and uniform illumination. The steers freely moved in the pens during the LMD measurement. One LMD measurement day lasted 21 h and consisted of 10 consecutive one–hour rounds of data recording. Methane concentrations in exhaled air were measured twice per animal during each one–hour round, corresponding to an approximate time slot of 10 minutes per animal, followed by a 20-minute break before its next measurement. However, these 10-minute slots also included the time required to switch between animals and to open a new data file on the LMD. The actual effective measurement time per animal and replicate was therefore approximately 7 to 8 minutes.

Each one–hour round of measurements was followed by a one –hour break to transfer LMD data to a laptop and recharge the battery. During the <10-minute measurement interval, the operator pointed the green laser beam of the hand-held LMm-G (Crowcon, Tokyo Gas Engineering Co. Ltd.) at the nostril area of the respective steer. Point measurements of CH_4 concentration in the exhaled air were recorded in ppm-m at an interval of 0.1 s, yielding 600 data points per minute. Background ambient CH_4 was accounted for in the measured values using the offset function of the LMD. For all measurements, a distance of 1 m was allowed between the animal's nostril area and the LMD. The operator continuously monitored the distance using a laser distance meter (Bosch, Stuttgart, Germany) held in the left hand, while the LMD was held in the right hand. The methane data were transmitted via Bluetooth to an Android mobile phone (placed in a work coat pocket) and stored on its memory card before being downloaded to the laptop. To account for variation in distance and nostril movement, the operator used a second mobile phone (placed in the second coat pocket) to record voice notes indicating "in" and "out" positions, as well as the "start" and "end" of each measurement. "In" was defined as 1 m of distance and the laser pointing to the nostril, while "out" was a moment when the beam could not point to the nostril area or the distance was not 1 m. "Start" was the moment when the operator started a measurement cycle while "end" was the time when the operator pressed the stop button of the LMD. The LMD and the Bluetooth–paired Android phone were synchronized to the exact same second, as was the second phone used for voice recording. Because the wireless internet connection on site was stable, all devices automatically synchronized their clocks to local time upon startup; this ensured that the LMD measurements aligned precisely with the timestamps in the audio recordings.

2.4 Data preparation

The LMD measurement data were prepared for statistical analysis using the following data cleaning steps (Supplementary Resource S2). In step 1, all 1236 raw data files of <10-minute LMD measurements were cleaned of "out" positions based on the voice recording data by matching the time stamps of voice recording and LMD data. In step 2, only LMD measurements recorded between 10:00 a.m. on the first day and 07:00 a.m. on the subsequent day were retained. In step 3, any <10–minute LMD file containing ≤1500 data points was excluded. This threshold was based on the assumption that with an effective measurement time of 7 to 8 minutes per animal and replicate, at least 5 minutes of valid LMD data should remain after removing "out" position segments. With the LMD's sampling frequency of 0.1 s, a 5–minute set of valid data should contain 3000 data points. The cutoff of 1500 data points represented 50 % of this expected minimum and was defined as the lowest acceptable level for inclusion. The third cleaning step thus resulted in 1002 LMD measurement files kept for analysis. In step 4, the arithmetic mean CH_4 concentration (ppm) of each <10-minute measurement was calculated. For step 5, seven 3-hour periods, namely 10:00 a.m. - 12:59 p.m. (P1), 1:00-3:59 p.m. (P2), 4:00-6:59 p.m. (P3), 7:00-9:59 p.m. (P4), 10:00 p.m. - 00:59 a.m. (P5), 01:00-03:59 a.m. (P6), and 04:00-06:59 a.m. (P7) were defined. For measurements in each period and day, the arithmetic means of the respective LMD measurements ($1 \leq n \leq 6$ measurements) were averaged per animal and experimental phase. The final data preparation step (step 6) included the calculation of Z scores based on mean CH_4 per FG and its standard deviation; only <10-minute measurements with an absolute Z score < 1.5 were retained for statistical analyses. Given two eructations on average within the expected 5–minute period of valid data, their influence on the mean or the Z scores was expected to be minimal. Moreover, none of the six data cleaning steps specifically targeted extremely high or low values.

To compare the LMD with the chamber, the mean CH_4 concentration within each 3-hour period (P1-P7) was calculated for the chamber data sets per animal and measurement phase; comparisons were made between chamber CH_4 and LMD CH_4, bearing in mind that the two instruments were used on consecutive days.

2.5 Data analysis

Descriptive statistics were calculated for the means of CH_4 records, expressed in ppm, obtained from LMD measurements and

in the respiration chamber, using R 4.0.3. The Tukey *post hoc* test was applied to compare differences among means (*multcomp* package) (R Core team, 2019) and the level of significance was determined at $P < 0.05$. Intra-class correlations (ICC) were calculated using a two-way mixed-effects model and the absolute agreement (Koo and Li, 2016) within the ICC function of the *psych* package (Revelle, 2024) to assess the agreement between repeated measures of each period across all measurement days within the same animal.

The Spearman correlation between the mean CH_4 concentration of the LMD and chamber measurement was calculated with the *R Stats* package (R Core team, 2019).

Non-parametric correlation analysis and a bootstrapping regression model with R = 3000 replication runs (boot function in the *boot* package v1.3-27) (Canty and Ripley, 2021) was used to evaluate the effect of total rain (mm), mean relative humidity (%), mean temperature (°C), solar radiation (W/m^2) and wind speed (m/s), obtained from the research centers' meteorological station (Supplementary Resource S3), on mean LMD CH_4 concentration.

The influence of the animals' liveweight (LW) and of feed dry matter intake (DMI), crude protein intake (CPI), and acid detergent fiber intake (ADFI), both in grams per day and in grams per kilogram of metabolic weight (MW, $LW^{0.75}$), on the mean daily LMD CH_4 concentration were assessed using linear regression and correlation analysis. Mean LW (kg) and DMI, CPI and ADFI (all in g/kg MW) were compared among FG by variance analysis, whereby no FG effect on LW and intake values could be observed (Table 2). Furthermore, a linear mixed effects model with FG and period as fixed effects and animal as a random effect was fitted on the data, using the *lmer* function (R Core team, 2019). Measurement days and measurement phases were different for individual animals but had no effect on chamber CH_4 and LMD CH_4 concentrations; they were thus excluded from further statistical analysis.

3 Results

Descriptive statistics of the CH_4 concentrations for the respiration chamber and LMD measurements are presented in Table 3. Overall, the LMD measurements yielded lower means and higher variances than the CH_4 measurements in the chambers. The ICC estimate indicated a poor test-retest reliability of LMD measurements (0.37; 95 %-confidence interval $0.26 < ICC < 0.48$). No significant relation was found (adjusted $r^2 = 0.01$, $p = 0.33$) between the mean daily LMD CH_4 and LW, total DMI, total CPI, and total ADFI of the animals. Similar results were obtained from linear regression analysis between mean daily LMD CH_4 and DMI, CPI, and ADFI per kg MW, as well as correlation analysis for total dry matter and nutrient intake (g per animal per day) and dry matter and nutrient intake per kg MW.

TABLE 2 Mean (± standard error of the mean, SEM) of liveweight (LW; kg), dry matter intake (DMI), crude protein intake (CPI), and acid detergent fiber intake (ADFI) of animals in three different forage groups; all intake values are in $g/kg^{0.75}$ LW.

Variable	n	LW	DMI	CPI	ADFI
Overall mean	18	240 ± 6.8	91.0 ± 3.40	8.0 ± 0.43	38.7 ± 1.36
Forage group					
Brachiaria grass	6	242 ± 13.7	90.4 ± 5.55	7.8 ± 0.63	37.8 ± 2.25
Napier grass	6	255 ± 10.5	89.3 ± 5.49	8.3 ± 0.63	38.4 ± 2.39
Rhodes grass	6	223 ± 8.3	93.1 ± 7.46	7.8 ± 1.05	40.0 ± 2.72
p-value		0.16	0.91	0.90	0.81

n, number of animals; for neutral detergent fiber (NDF) intake see Korir et al. (2022).

The Spearman correlation between the LMD and the chamber measurements was weak ($r_s = 0.29$), but significant ($p < 0.001$) (Figure 1). On average, the mean LMD CH_4 was 83 ± 9 % lower than the corresponding mean chamber CH_4.

Non-parametric correlation analysis revealed that mean LMD CH_4 was related to rain (Kendall's $\tau = -0.15$; $p < 0.01$), relative humidity (Kendall's $\tau = -0.20$; $p < 0.001$), temperature (Kendall's $\tau = 0.16$; $p < 0.01$), wind speed (Kendall's $\tau = 0.25$; $p < 0.001$), and solar radiation (Kendall's $\tau = 0.28$; $p < 0.001$). Both mean chamber CH_4 and mean LMD CH_4 were affected by period ($p < 0.001$), while FG had no effect ($p > 0.05$) (Table 3). In the chamber, the highest mean CH_4 was measured in P2 and P3. Similarly, mean LMD CH_4 was highest during P1 to P3. For both measurement setups, the CH_4 concentrations then gradually decreased until P7 (Supplementary Resource S4).

Furthermore, a significant interaction effect between FG and period on the mean CH_4 concentrations (Table 3) was observed for the chamber measurements ($p < 0.001$) but not for LMD measurements. The mean chamber CH_4 for steers fed Rhodes grass was lower in P2 and P3 and decreased less sharply between P3 and P4 than for steers fed Brachiaria or Napier grass. Conversely, in steers fed Rhodes grass the decrease in mean chamber CH_4 between P2 and P3 was greater than the corresponding decrease in steers fed Brachiaria grass (Supplementary Resource S4).

Comparing the rankings of the three FG based on their overall mean CH_4 concentrations with both measurement approaches, we found a full agreement between the rank of chamber CH_4 and LMD CH_4 (Table 3), and also a relatively strong agreement between the chamber CH_4 and LMD CH_4 ranks of periods P1 to P7 (Kendall's $\tau = 0.62$, $p = 0.069$). In contrast, the correlation between the rank of overall mean chamber CH_4 and mean LMD CH_4 of individual animals (Figure 2) was weak (Kendall's $\tau = 0.19$, $p = 0.293$).

4 Discussion

This study assessed the ability of the LMD to capture diurnal CH_4 concentration profiles and rank mean exhale air CH_4 concentrations across three different forage groups, 3-hour periods within a day, and individual animals in a typical tropical open barn setting. Repeated respiration chamber measurements of the same animal, but from the previous day, served as reference for LMD measurements in an open pen of the subsequent day. This aspect has to be kept in mind when discussing the differences between chamber and LMD measurements, both in absolute and relative terms.

TABLE 3 Mean (± standard error of the mean, SEM) methane (CH_4) concentrations and ranks for the respiration chamber (chamber) and the laser methane detector (LMD), overall, by forage group (FG), and by 3-hour period (period).

		CH_4 concentration (ppm)		Rank	
Factor	n	Chamber	LMD	Chamber	LMD
Overall mean	316	128.5 ± 1.93	18.9 ± 0.43	–	–
FG					
Brachiaria grass	104	129.6 ± 2.96	18.9 ± 0.78	2	2
Napier grass	104	141.1 ± 3.69	20.6 ± 0.74	3	3
Rhodes grass	108	115.2 ± 2.88	17.1 ± 0.66	1	1
Period					
1 (10:00 a.m – 12:59 p.m.)	29	143.4^{d} ±4.40	23.8^{c} ±1.66	5	7
2 (1:00 – 3:59 p.m.)	41	158.8^{e} ±4.87	23.5^{c} ±1.47	7	6
3 (4:00 – 6:59 p.m.)	51	156.5^{e} ±4.52	20.7^{bc} ±1.16	6	5
4 (7:00 – 9:59 p.m.)	47	135.6^{d} ±3.78	16.9^{ab} ±0.81	4	3
5 (10:00 p.m. – 0:59 a.m.)	48	118.1^{c} ±3.21	16.6^{ab} ±0.87	3	2
6 (1:00 – 3:59 a.m.)	48	105.4^{b} ±2.58	17.3^{ab} ±0.91	2	4
7 (4:00 – 6:59 a.m.)	52	93.2^{a} ±2.30	15.9^{a} ±0.67	1	1
Effects					
FG		0.08	0.22	–	–
Period		<0.001	<0.001	–	–
FG*Period		<0.001	0.26	–	–

[a,b] Means in the same column with different superscript letters differ significantly ($p < 0.05$). n, number of observations.

Evaluating the quality of LMD measurements first, previous studies also reported low test-retest reliability of LMD measurements in the range of 0.03 to 0.07 (Pickering et al., 2015), 0.23 (Rey et al., 2019), and 0.52 to 0.59 (Niero et al., 2020). With an ICC of 0.37, our results fall in the middle of that reported range. Similar to the findings of the associated chamber measurements, LMD measurements were not affected by LW, total DMI, CPI, and ADFI (Korir et al., 2023). This can be attributed to the overall setup of the feeding experiment which provided the basis for the present LMD *versus* chamber comparison and aimed to evaluate three irrigated forage species for year-round green fodder supply in small-scale African zero-grazing systems. Hence, the tested forages were chosen based on their potential to support animal growth, leading to similar ADF concentrations (Korir et al., 2023). Furthermore, all animals were fed at 110 % of their individual voluntary feed intake, with daily adjustments based on actual consumption. Consequently, average DMI (89 to 93 g DM/$kg^{0.75}$) was highly consistent across the three FGs. The narrow range in ADF concentrations across FG as well as in DMI and ADFI across animals explains the absence of a significant effect of these variables on the measured methane

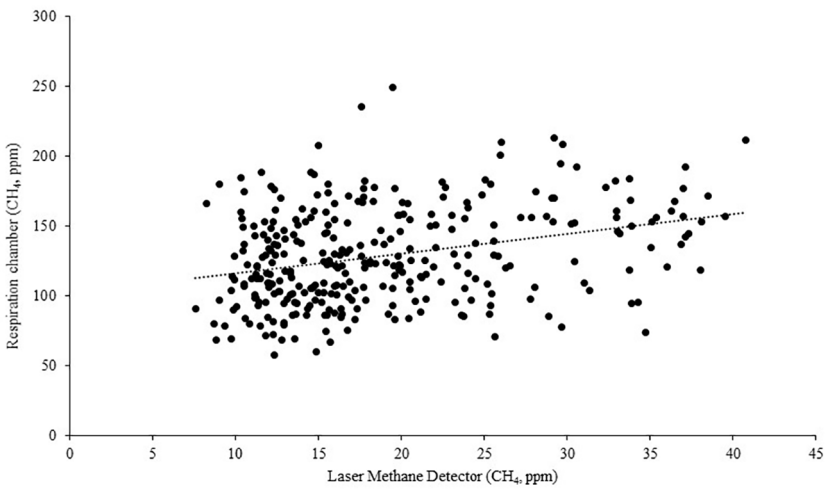

FIGURE 1
Relationship (r_s=0.29, $p < 0.001$) between methane (CH_4) concentration measurements with the laser methane detector and the respiration chamber.

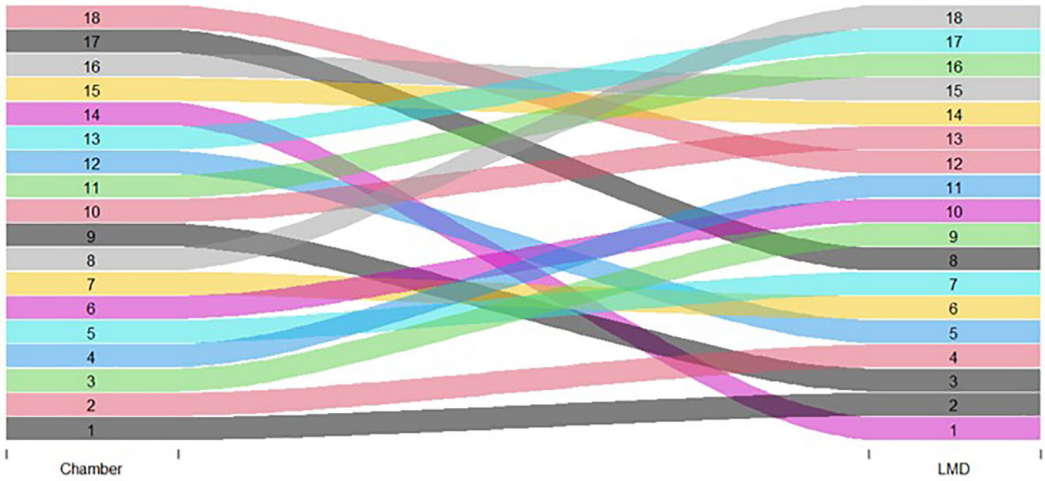

FIGURE 2
Alluvial diagram of the ranks (numbers 1 to 18) of eighteen individual animals based on mean methane (CH_4) concentrations measured in the respiration chamber (chamber) and with the laser methane detector (LMD).

emissions (Korir et al., 2023). In contrast, Rooke et al. (2014) reported a significant influence of DMI on LMD readings; however, their study involved one ration containing 50 % roughage and another containing < 10 % roughage, resulting in a wide variation in feed intake levels that may explain the differing outcomes.

On average, the LMD CH_4 values were 83 % lower than the chamber CH_4 concentrations, and with a coefficient of 0.29 the correlation between LMD CH_4 and chamber CH_4 was low, though significant. This result aligns with reported correlation coefficients in the range of 0.18 and 0.39 (Ricci et al., 2014; Denninger et al., 2020), but is lower than correlation coefficients > 0.5 reported by several other authors for cattle (Chagunda and Yan, 2011; Chagunda et al., 2013; Sorg et al., 2018a; Kobayashi et al., 2021b) and sheep (Doran, 2015). The different feeding schedules in the chamber, where all feed was offered in one meal in the morning, and in the pen on the following day, where the daily amount of forage was portioned into three meals, may have been a main reason for the low correlation, as feeding frequencies affect CH_4 emission patterns (Rooke et al., 2014).

Furthermore, disturbance of the LMD readings by environmental variables such as air temperature, humidity, (solar) light intensity, wind speed, and aerosol contaminations (Chagunda et al., 2013; Sorg et al., 2018a; Zhao et al., 2020) may also have played a role. LMD measurements in naturally ventilated sheds yielded a low correlation (r = 0.12) with chamber data for freely roaming ewes but a higher value (r = 0.53) for restrained steers (Ricci et al., 2014). Several other outdoor studies (Chagunda, 2013; Chagunda et al., 2013; Sorg et al., 2018b; Pinto et al., 2020) also reported a significant influence of atmospheric and light conditions on LMD measurements. While high air humidity can increase the stability of LMD measurements (Chagunda et al., 2013), increased air movement can dilute the exhale air CH_4 column (Chagunda et al., 2013; Sorg et al., 2018b). In the present study, the non-parametric Mann-Kendall monotone trend analysis detected significant effects of rain, ambient temperature, relative humidity, wind speed, and solar radiation on the LMD measurements. These results indicate that the multiple data cleaning steps performed prior to the statistical analysis of LMD data (Supplementary Resource S2) did not fully mitigate atmospheric and light disturbances. Independent of this, a general aspect to be considered in using the LMD is that time for data cleaning and preparation is substantial before proper data analysis and statistical evaluation can be undertaken (Ricci et al., 2014; Kobayashi et al., 2021b; Sorg, 2022), which is also reflected in the six subsequent data cleaning steps undertaken in the present study.

The overall mean concentration of 18.9 ppm CH_4 measured with the LMD was within the range of means reported for South African Bonsmara (*B. taurus* x *B. indicus*) heifers (15.3 to 32.7 ppm) grazing different types of natural or sown pastures (Grobler et al., 2014), and respiration means reported for growing Hanwoo (*B. indicus*) steers on three diets varying in roughage (≤35 %) to concentrate ratios (Kang et al., 2022).

Several studies suggested the suitability of the LMD to capture activity-dependent CH_4 profiles (Chagunda et al., 2013; Roessler and Schlecht, 2021; Boré et al., 2022). When analyzing the seven 3-hour periods from morning feeding (P1) to the end of the night resting phase (P7), distinct periodic changes in CH_4 concentrations were observed for both LMD and chamber measurements (Table 3 and Supplementary Resource S4).

Although the rankings of the two measurement approaches did not fully converge, ranks 1 to 3 were in both cases assigned to the hours of active feeding and intermittent rumination (from 10 a.m. to 6:59 p.m.), while ranks 4 to 7 were attributed to the evening and night resting and rumination phase (Table 3). These patterns are consistent with observations from a detailed sheep study. In that study, LMD CH_4 concentrations peaked 40 minutes after the morning feeding, exceeding levels recorded during midday rest and a less intense afternoon feeding period (Doran, 2015). However, while in the present study the daily feed ration was offered in one meal at the beginning of a 22.5-hour measurement cycle (at 10:00 a.m., i.e., the start of P1) in the respiration chamber, the three meals offered in the pen were starting at 9:00 a.m. (one hour before P1), 12:00 p.m. (toward the end of P1), and 4:00 p.m.

(start of P3). Although within a 24-hour period the same amount of feed was offered in both cases, this resulted in distinctly different diurnal CH_4 profiles between the two measurement approaches, with a prolonged high CH_4 concentration plateau in the chamber until the end of P3, especially for Brachiaria and Napier grasses, and a more rapid CH_4 concentration decrease in the pen during P2, followed by slight CH_4 increases in P3 (Napier grass), P5 (Brachiaria grass), and P6 (Rhodes grass), respectively. Similar CH_4 concentration patterns associated with increased microbial fermentation activity after a meal have been reported from LMD measurements in sheep and cattle (Ricci et al., 2014; Doran, 2015; Kobayashi et al., 2021b).

Despite the lack of significant differences in mean LMD CH_4 values among the three FG and their similar ADF concentrations, the three forages were ranked identically by both the chamber and the LMD, as also reported by Grobler et al. (2014) and Ricci et al. (2014). The significant interaction between FG and period for chamber CH_4 concentrations might nevertheless be due to the slightly higher ADF concentration in Rhodes grass compared to Brachiaria and Napier grasses. The chamber CH_4 concentration data reveals pronounced CH_4 peaks during periods P2 and P3, followed by a fast decline (Supplementary Resource S4), likely due to a high fermentation rate of Brachiaria and Napier grasses. The slower increase in CH_4 concentration from P1 to P4 and subsequent more gradual decline in animals fed Rhodes grass may be due to its slower microbial fermentation, likely caused by its 9-12% higher ADF content compared to Napier and Brachiaria grass (Castro-Montoya et al., 2021).

In contrast, ranking individual animals by their overall mean CH_4 concentration in exhaled air as measured with the LMD showed only partial agreement with the respective ranking derived from the chamber measurements. Six animals were ranked very similarly, differing by only one or two rank positions, and the ranks of three animals were somewhat similar, differing by 3 or 4 positions. When classifying the 18 animals as high, medium, and low emitters based on the chamber CH_4 concentrations (6 animals per cluster), 3, 2, and 3 animals, respectively, were correctly assigned to their corresponding cluster. The number of overall LMD data points retained after the six cleaning steps (Supplementary Resource S2) does not explain the discrepancies of five or more rank positions between chamber and LMD found for nine animals. Of these animals, two had fewer than 200000 data points in the cleaned LMD dataset, three had between 200000 and 250000 data points, and four had more than 250000 data points. The latter included animal BN08, which had the greatest rank difference (chamber rank 14, LMD rank 1) with 255755 LMD data points.

It is important to note that the current study design had several limitations. With only six animals per feeding group (FG) and 18 animals in total, the sample size was smaller than that reported in other LMD studies. However, Grobler et al. (2014) also used only four cattle per each of four treatment groups in their grazing trial. In contrast, other studies focusing on animal nutrition had a sample size of around 30 animals (Doran, 2015; Kang et al., 2022). Another major issue was the discrepancy in feeding schedules on the measurement days in the chamber, where all feed was offered in one meal in the morning, and in the open pen on the following LMD-measurement day, where the total feed offer was divided into three portions distributed in the morning, at noon, and in the afternoon. This undoubtedly influenced the diurnal CH_4 emission patterns (Rooke et al., 2014). However, animal behaviour observations were lacking, which would have allowed a clear identification of eating, resting, and ruminating periods in both setups. In addition, the three tested forages were of similar quality, particularly in terms of ADF content, and *ad libitum* feeding resulted in DMI levels that were very similar across forage groups. A third limitation, which has already been mentioned, was that chamber measurements under fully controlled conditions always preceded the open –pen LMD measurements by one day. This introduced day–to–day variation at the individual animal level and, naturally, differences in environmental conditions.

5 Conclusions

To date, only five studies have reported the use of the LMD to measure exhaled CH_4 in tropical ruminant systems. While these studies concluded that the LMD is suitable for assessing management impacts on CH_4 emissions, our results indicate that some caution is warranted. Although the LMD is easy to use, highly mobile, and affordable, the considerable effort required to clean its raw data sets - an essential step we strongly recommend - diminishes its practical advantages.

Additionally, concerns about the instrument's suitability for methane inventories arise from its sensitivity to atmospheric and light effects, which our data cleaning protocol could not eliminate. While previous studies used LMD measurements to identify high and low methane-emitting ruminants, our study was less successful in ranking individual animals based on their exhaled methane concentrations. This may be due in part to the relatively small number of animals in this feeding study as compared to large-scale phenotypic assessments of methane emission levels. In contrast, ranking of different forages and of feeding and rumination/resting periods during a day by their average methane concentrations was more successful. Despite the limitations of our experimental setup that affected the results, standardizing not only the data collection but also the data analysis protocol for LMD measurements would facilitate the use of this device for assessing CH_4 emissions from ruminants under tropical on-farm conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by the International Livestock Research Institute, Nairobi, Kenya. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owner (ILRI), for the participation of their animals in this study.

Author contributions

AA: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. RR: Data curation, Formal analysis, Supervision, Validation, Visualization, Writing – review & editing. DK: Data curation, Investigation, Methodology, Writing – review & editing. PS-S: Data curation, Writing – review & editing. JG: Conceptualization, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – review & editing. LM: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. ES: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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