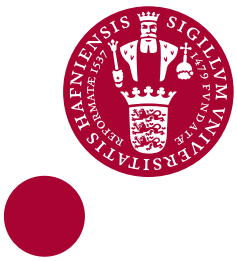

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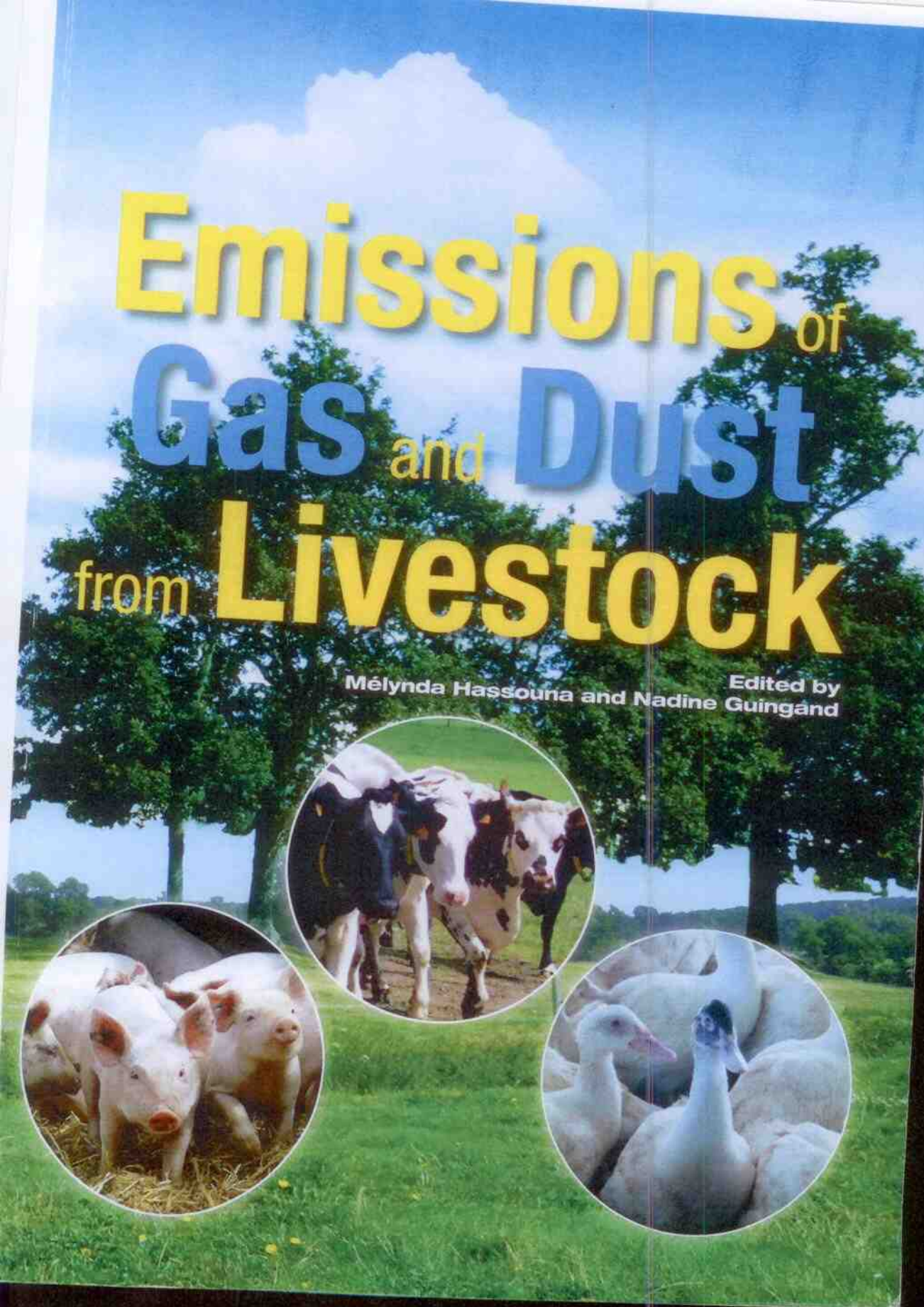

COMPARISON OF METHANE EMISSIONS FROM CATTLE ASSESSED BY THREE DIFFERENT METHODS: OPEN-CIRCUIT RESPIRATION CHAMBERS, IN VITRO GAS PRODUCTION AND THE CO_2-METHOD

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ABSTRACT: Different methods exist for estimating methane production by ruminants with different optimal applicability. The objective of this study was to compare the estimates of methane emissions from cattle resulting from 3 different measuring techniques: Open-circuit respiration chambers (RESPT), In vitro gas production (IVGPT) and the CO_2-technique (CO_2T). The techniques were applied in three separate experiments but with the exact same feed rations containing 35% DM of wheat (W), Molasses (M) or molasses+0.9%DM sodium bicarbonate (MBic). Significant differences were found between methods when comparing the values of ml CH_4/g DM. The respiration chambers gave the highest values and IVGPT the lowest. Within the IVGPT and RESPT experiments, significant differences were found among the three rations, with W giving less CH_4/g DM than molasses rations. For CO_2T, the same numerical ranking was observed but the differences were not significant. The residual model errors were of the same magnitude for all three methods. It is concluded that the absolute values of CH_4 production differ significantly among the three experiments. This may be caused by the measurement techniques or/and the differences in cows. The ranking of rations (W<M=MBic) was the same for all methods.

Keywords: CH_4, cattle, measuring method, comparison

INTRODUCTION: Numerous methods have been developed to estimate the actual emissions of livestock and evaluate potential methods for methane mitigation. They are based on different principles and have different optimal applicability (Storm et al., 2012). Two relatively new approaches for estimating methane emission from ruminants are modified *in vitro* gas production (IVGPT) techniques (Bhatta et al., 2008) and the CO_2-technique (CO_2T) (Madsen et al., 2010). These methods are fundamentally different from the traditional open-circuit respiration chamber technique (RESPT): IVGPT simulates the ruminal fermentation of feed under controlled laboratory conditions, while CO_2T makes spot measurements of the CH_4/CO_2 ratio in the exhaled air of ruminants and multiplies it with the estimated total CO_2 production. Few studies have been published on the comparison of these new methods with RESPT. Comparing methods used in separate studies is complex due to differences between feeds and animals used in the experiments. The aim of this study was to compare the estimates of methane production resulting from three individual experiments employing RESPT, CO_2T or IVGPT, but with the exact same feed rations.

1. MATERIALS AND METHODS: Three separate experiments were conducted employing each method and different animals. The same three feed rations were used in all experiments. They all consisted of grass-clover silage (49% of dry matter (DM)) and soy bean meal (14 % of DM) supplemented with 35 % (DM) of either crushed wheat (W), sugar beet molasses (M) or sugar beet molasses with sodium bicarbonate (0.9 % of DM) (MBic). The chemical composition of the rations is presented in Hellwing et al. (2012). All portions of the feed rations were mixed from the same

batches of ingredients at the experimental farm facilities of Aarhus University, Foulum, Denmark. All gas volumes are reported at standard temperature and pressure (0°C, 100 kPa).

The RESPT experiment included a fourth ration where the 35 % (DM) supplement was sodium-hydroxide treated wheat. It was conducted according to a 4x4 Latin Square design with 4 lactating Holstein-Friesian cows. The average body weight (±SD) was 570±36 kg, average dry matter intake (DMI±SD) 18.0±1.6 kg/d, and average milk yield 21.4±6.1 kg ECM/d. The mixed rations were prepared once daily and fed ad libitum with two feedings a day. In each period the cows were subjected to a 3-week adaptation period before methane emission was measured in open-circuit respiration chambers over 4 consecutive days. For a detailed description of the experiment see Hellwing et al. (2012).

The CO_2-technique was applied to 3 Dexter heifers in conjunction with measurements of their CO_2-production in traditional open-circuit respiration chambers. The experiment was conducted as a 3x3 Latin Square with 3 periods consisting of 2 weeks' adaptation followed by one week where measurements were conducted. Each animal was monitored with CO_2T for one 22 h period. All feed for the entire experiment was prepared once from the same batches of ingredients as used in the RESPT experiment, and at the same facilities. The TMRs were immediately vacuum-packed in portions for 1 day, frozen, and transported to the University of Copenhagen. Each portion was thawed at room temperature overnight before being feed ad libitum with one daily feeding. The average BW (±SD) of the heifers was 226±11 kg and the average DMI (±SD) was 5.1±0.3 kg/d. For further description see Haque et al. (2012).

For IVGPT, feed samples from the CO_2T-experiment were dried (60°C) and milled (1 mm mesh; Cyclotec 1093 sample mill, Foss Analytical, Hilleroed, Denmark). Portions of 0.500±0.01g of feed were weighed into F57 filter bags (Ankom Technology, Macedon, NY, USA). After sealing, the filter bags were put into 100 ml Duran bottles fitted with automatic wireless *in vitro* gas production modules (Ankom Technology, Macedon, NY, USA). Rumen fluid was obtained from two rumen fistulated jersey heifers at UCPH. The heifers were on a diet of hay supplemented with grazing. Buffered rumen fluid inoculum was prepared according to the directions of Menke and Steingass (1988). Portions of 90 ml mixed inoculum fluid was added to each IVGPT module, which were closed, fitted with evacuated airtight gasbags (FlexFoil, 1 L; SKC Ltd, Dorset, UK) on the outlets and incubated at 39°C for 48 hours with gentle stirring (20 rpm). Gas pressure was detected every 5 minutes and gas released when the pressure exceeded 3.45 kPa above atm. pressure. After incubation, the volume of total gas produced was calculated by applying the ideal gas law. The percentage of CH_4 in the released gas was measured by gas chromatography (Agilent 7820A GC, Agilent Technologies, Santa Clara, USA; equipped with a TCD detector, a HP-PLOT Q column (30 m x 0.53 mm x 40 µm) and employing H_2 as carrier gas).

Each experiment was analyzed with individual statistical models to account for individual study design: RESPT by a MIXED model in SAS with carbohydrate source, ration pH, carbohydrate*pH interaction and period as fixed factors and cow as random factor (Hellwing et al. 2012). CO_2T was analyzed by a GLM model in SAS with cow, period and ration as fixed effects and by a MIXED model differing only in cow as a random factor. For IVGPT, the effect of ration was evaluated by a linear model in R combined with multiple comparisons of means by Tukeys contrasts. Methods were compared within each ration by the same approach in R. Significance level was set at $P<0.05$.

2. RESULTS AND DISCUSSION: The raw values for ml CH_4/g dry matter, as assessed by each of the methods, are plotted in Figure 1, and the mean values including results of the statistical comparisons are presented in Table 1. For IVGPT, three values were omitted from the statistical analysis: two due to module failure during incubation; the third was assessed as an outlier on the basis of a Cooks distance above 0.5 combined with deviation from a normal distribution according to Shapiro-Wilk normality test in R.

All three methods resulted in lower values of CH_4 production per gram DM for the W ration than for the two molasses rations. There was a significant difference between starch-based and sugar-based rations in the RESPT and IVGPT experiments (P=0.03 and P<0.001). This supports other findings that starch results in less ruminal CH_4 than sugar. No significant differences were found between feed rations in the CO_2T experiment. This is probably due to the weak statistical strength of the 3x3 Latin Square design. The variation between heifers was almost as high as the variation between diets and no significant differences could therefore be observed.

The root mean square errors of the three statistical analysis (Table 1) are; however, of the same magnitude, indicating that the variation within experiments, caused by random variations, e.g. in the measurement instruments, are similar. The slightly higher RMSE for CO_2T can be explained by the partial sampling of exhaled breath with this technique (Haque et al., 2012). Additionally, is fairly easy to include more animals/units in both CO_2T and IVGPT experiments, making the statistical comparisons between treatments stronger.

Within each feed-type the comparison of methods showed significant differences. RESPT consistently gave higher estimates than CO_2T. For M and MBic, rations the difference was significant (P<0.01), although for W it was not (P=0.08).

Table 1. Mean values of ml CH_4/g DM (± standard deviation) for each combination of method and ration followed by results from the statistical analysis for effect of ration within methods.

Method	Mean ml CH_4/g DM			RMSE[a]	P_{ration}	$P_{carbohydrate}$
	Wheat	Molasses	Molasses+Bic			
RESPT	32.1	35.9	34.6	1.9	-[c]	0.03
IVGPT	15.8	23.8	24.7	1.5	<0.001	-
CO_2T	26.4	28.5	29.8	2.6	NS	-

[a]Root mean square error/residual standard error of the model used within each technique.
[b]This experiment was conducted as a 4x4 latin square with at fourth ration included.
[b] - = not applicable

CO_2T, in turn, gave significantly higher estimates for CH_4 production/g DM than IVGPT (P=0.004, 0.05, and <0.001 for W,M, and MBic). These differences may be due to other factors related to the individual experiments than the technique used for measuring CH_4-production. While care was taken to use the exact same feed rations in all experiments, there were large differences between the cows used and the experimental designs due to practical constraints. The relatively low gas production measured by IVGPT may also be related to the use of feed dried at 60°C (Parissi et al., 2005).

Figure 1. Production the CO_2-method (× respiration cha carbohydrate compo

CONCLUSION: In in a significantly l rations. Within feeds with RESPT > CO_2T must always be inter

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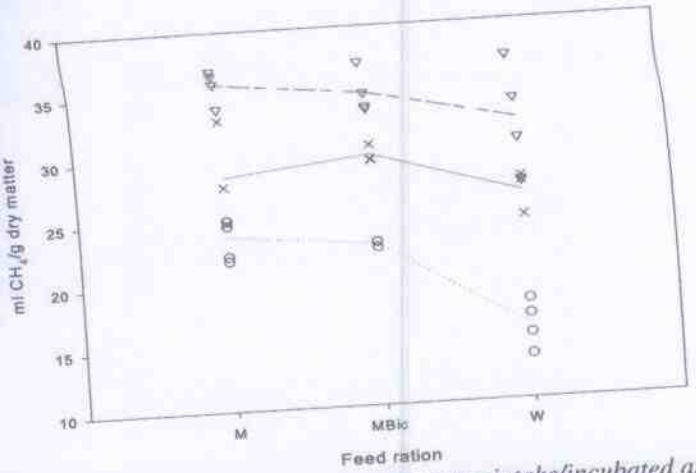

Figure 1. Production of methane per gram dry matter intake/incubated as assessed by the CO_2-method (×,—), in vitro gas production technique (○, ···) and open-circuit respiration chamber technique (▽, ---) for three cattle rations differing in carbohydrate composition of the concentrate. The lines connect the mean values for each method.

CONCLUSION: In two out of three experiments, the wheat-based ration(s) resulted in a significantly lower CH_4-production per gram DM than the molasses-based rations. Within feeds, the absolute values were significantly different among methods with RESPT > CO_2T > IVGPT. Therefore, absolute values obtained by any method must always be interpreted with care.

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