



The 75th EAAP Annual Meeting

1/5 September 2024 - Florence, Italy

Factors affecting the in vitro kinetics of total gas and methane in dairy cows using Gas Endeavour System

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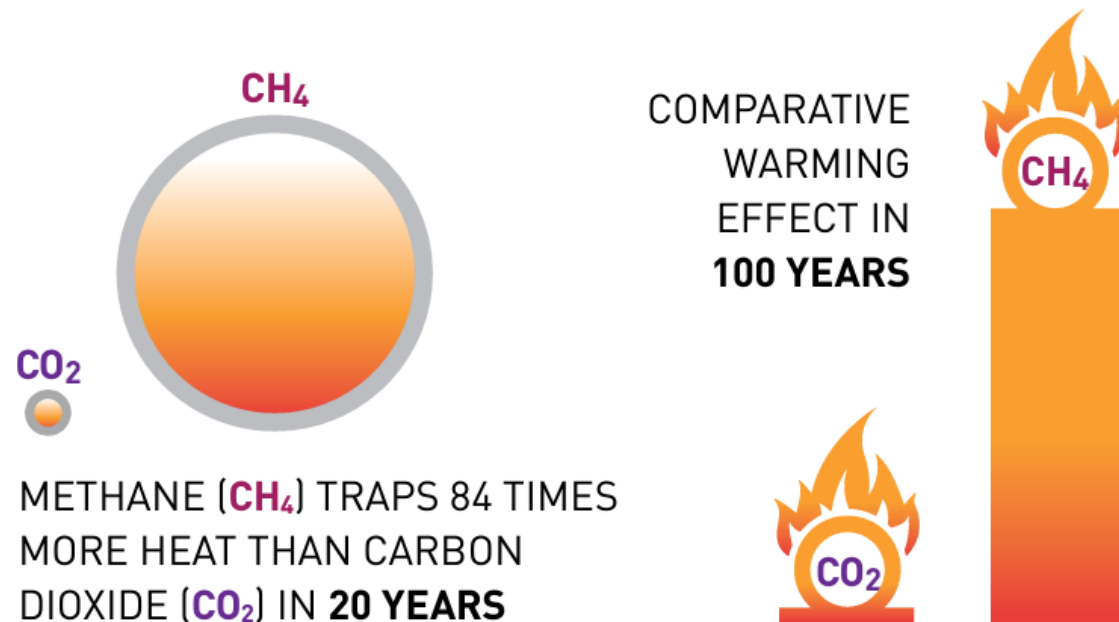
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Introduction

- Ruminants are responsible for 30% of global methane emissions (FAO,2020)
- Feeding manipulations
- Change in feeding during physiological stage
- Assessment of gas and methane production from ruminants
- *In-vitro* methods
- *In-vivo* methods

ENTERIC METHANE EMISSIONS





Objectives

- ◎ To standardize the procedure of the Gas Endeavour system for in vitro rumen fermentation
- ◎ To analyze the physiological stage and buffer as factors affecting the in vitro kinetics of total gas and methane production in dairy cows
- ◎ To predict the kinetics of gas and methane production using an exponential model

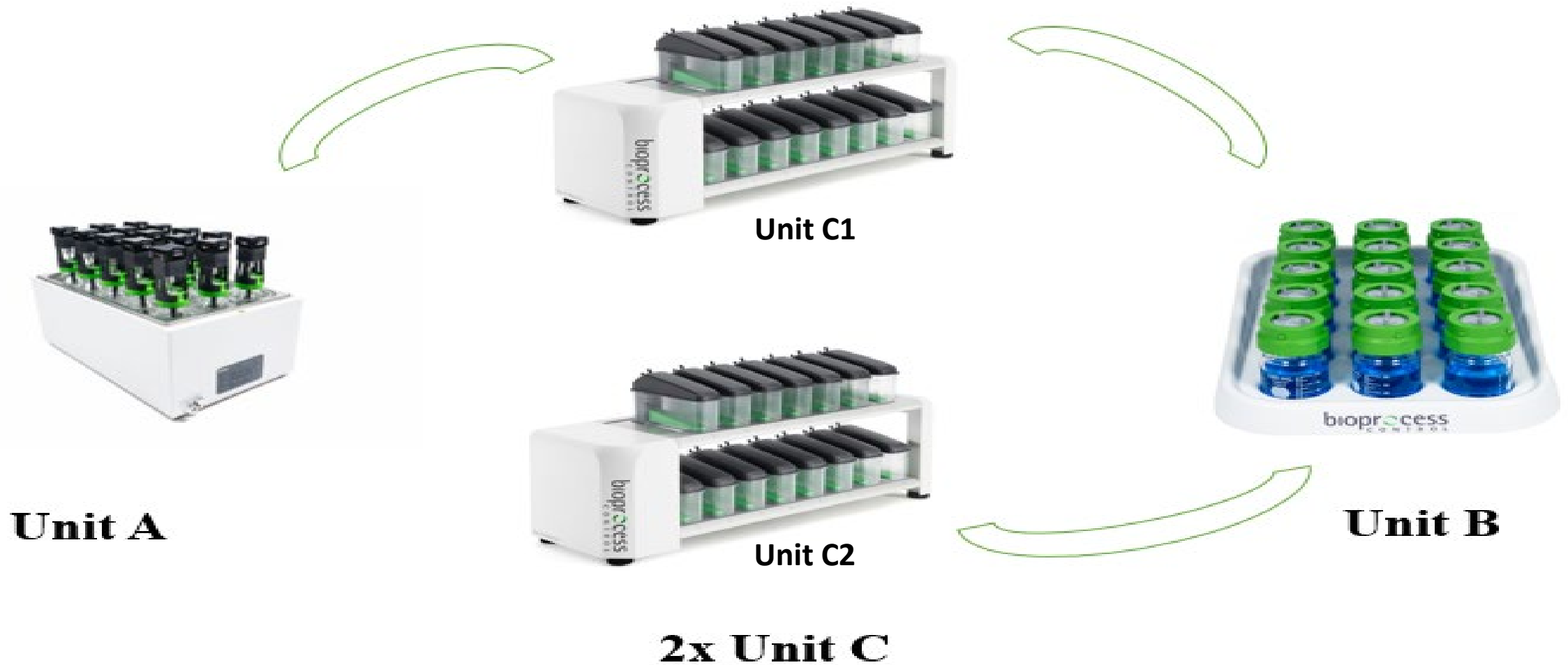


Materials & Methods

- 6 Simmental dairy cows: 3 lactating (93 ± 16 DIM) and 3 dry (247 ± 10 DIM) cows
- Rumen fluid collection (early morning)
- Buffered rumen inoculums were prepared using Menke (Menke *et al.*, 1979) and Van Soest (Goering & Van Soest, 1970).
- Rumen to buffered ratios are 1:2 and 1:4 respectively
- 2 incubation runs for total of 30 bottles
- 1g of dry and lactating diets were incubated

Ingredients	Dry	Lactating
DM	90.53	94.75
CP	15.01	16.1
EE	4.14	3.54
Ash	11.83	6.9
NDF	37.10	35.14
ADF	21.80	22.72
ADL	4.2	3.39
Starch	22.20	23.38

Materials & Methods





Materials & Methods

- 150 mL of inoculum used
- Anaerobic conditions maintained with CO₂
- Shaking water-bath (110 rpm) at 39°C for 24 h
- Gas volume measurement at 0°C, 1 atm and dry gas
- pH and VFA were measured after the end of incubation (data not shown)



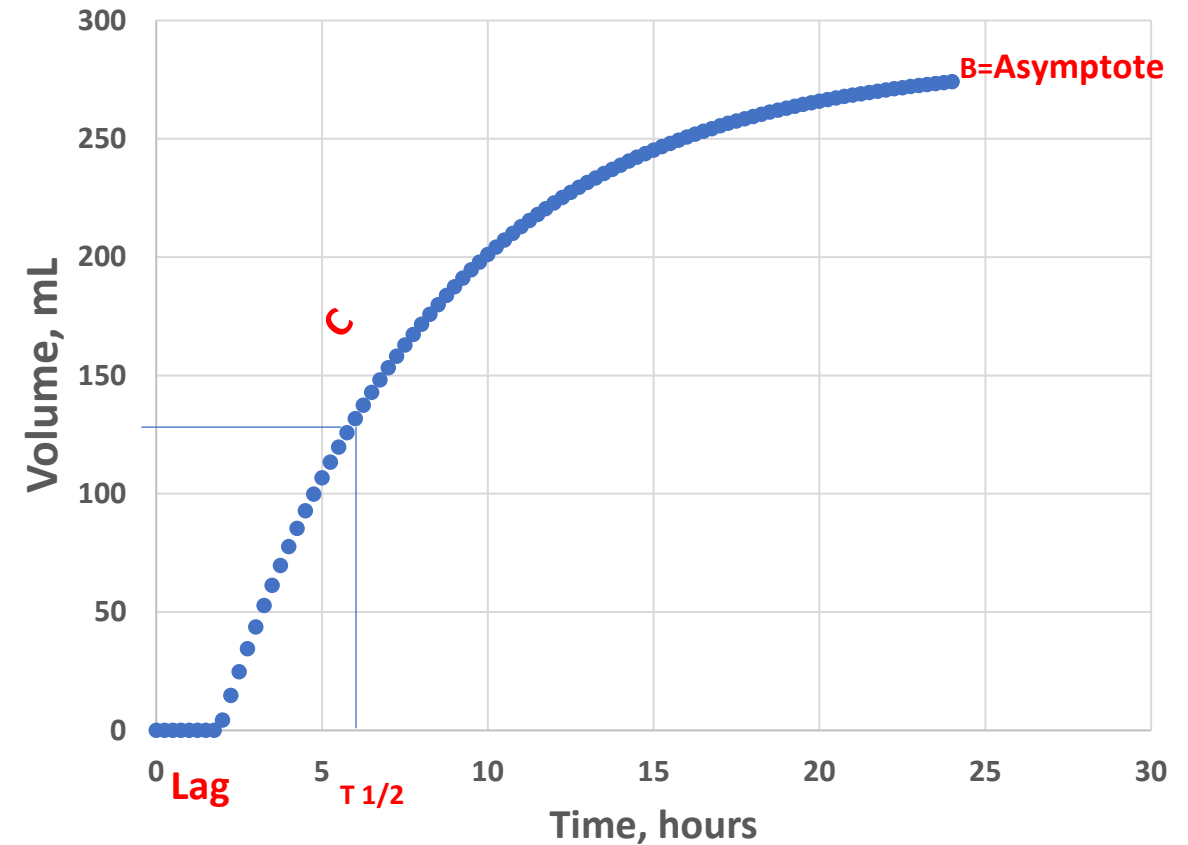


Materials & Methods

- Data was fitted with exponential model (Grings *et al.*, 2005)

$$y = B \times (1 - \exp^{-c \times [t - \text{lag}]})$$

- Where:
 - y is the cumulative volume of gas produced at time t (h)
 - B the asymptotic gas volume
 - c the rate constant
 - lag is the time (h) between inoculation and commencement of gas production





Materials & Methods

- All data were subjected to statistical analysis (R software v.4.1.1, R Core Team, 2021) using general linear model

where,

$$Y_{ijkl} = \mu + P_i + B_j + R_k + (P_i \times B_j) + (P_i \times R_k) + (B_j \times R_k) + \varepsilon_{ijkl}$$

- μ = overall mean
- P = effect of the cows physiological stage (i=2; D,L)
- B = effect of the buffer (j=2; M, VS)
- R= effect of run (k=2; 1,2)
- ε = residual

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Results



Results

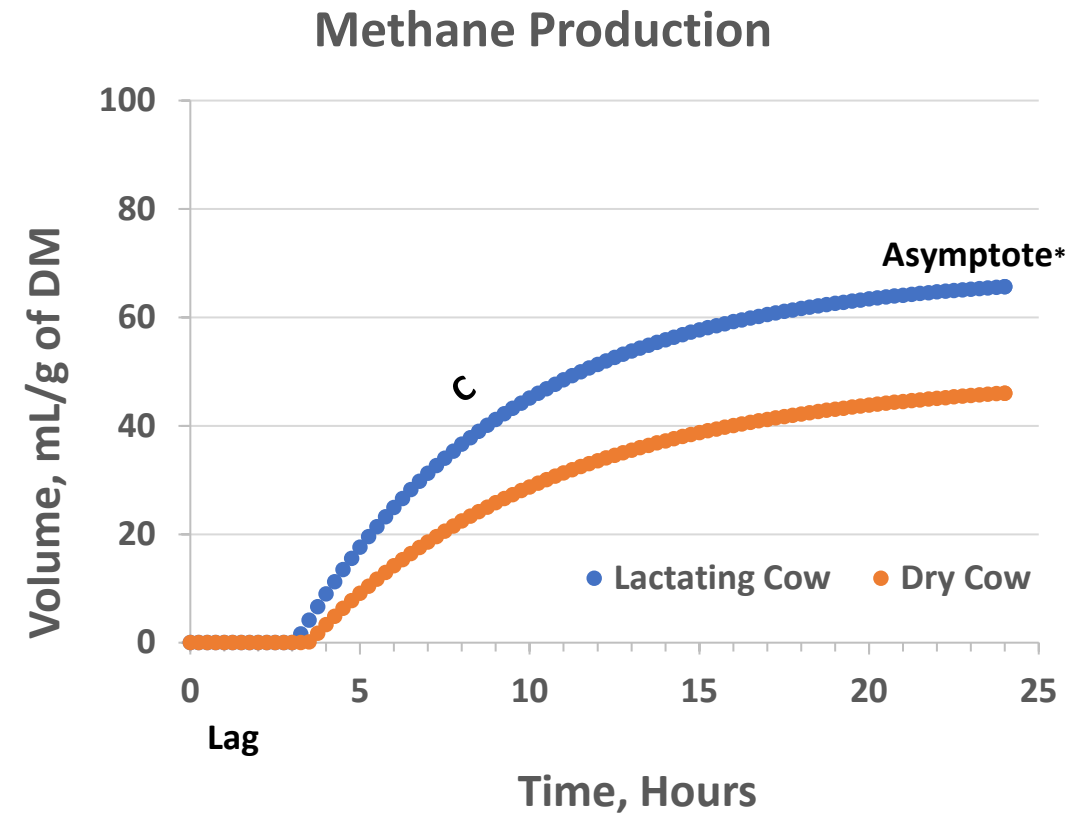
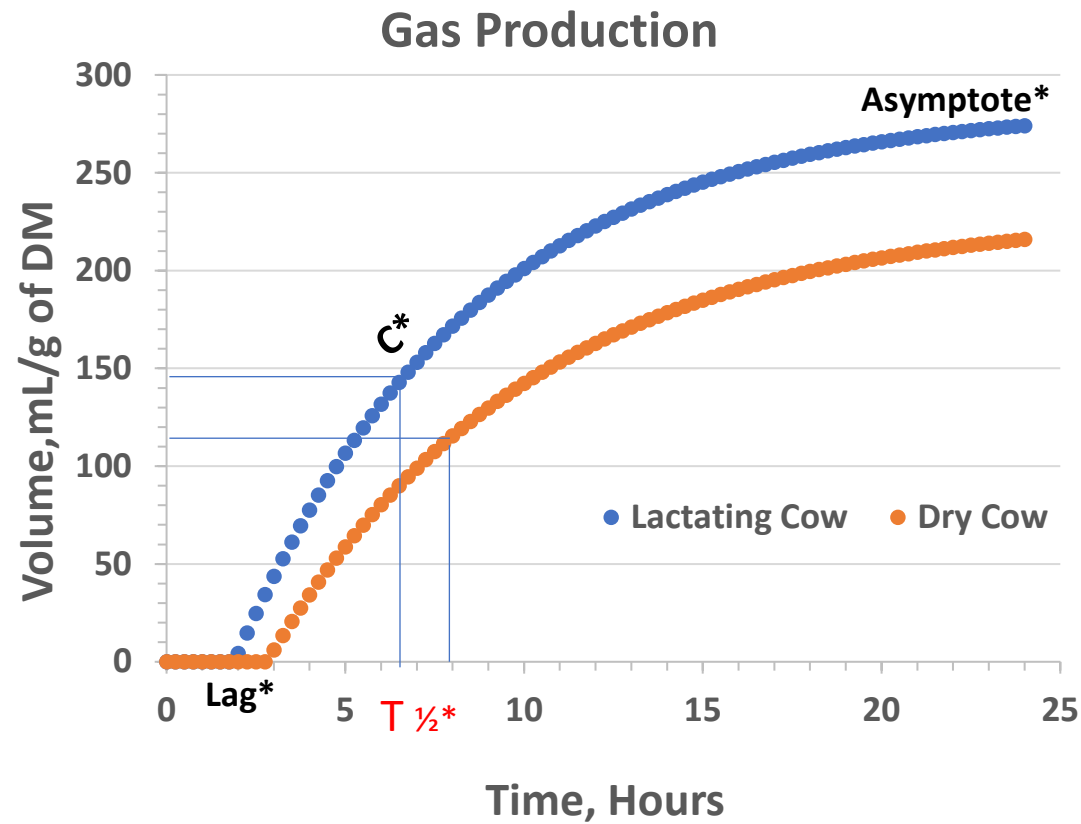
ANOVA reporting the F statistics of all the main effects and interactions

	Physiological Stage (PS)	Buffer system (BS)	Run (R)	PSx BS	PS x R	BS x R
Gas Production Kinetic Parameters						
B (ml/g of DM)	18.76***	13.86**	28.47***	0.06	5.29*	1.39
c (rate)	6.73*	1.27	2.26	0.00	4.69*	1.42
Lag (h)	5.94*	1.55	7.18*	1.53	1.05	0.00
T(1/2)	24.61***	0.36	5.61*	2.55	0.06	1.05
Methane Production Kinetic Parameters						
B (ml/g of DM)	14.35**	5.79*	49.31***	2.80	12.58**	4.71
c (rate)	1.16	0.67	35.7***	0.08	0.05	2.60
Lag (h)	0.04	0.14	1.59	0.05	2.30	0.09
T(1/2)	1.22	5.37*	15.09	0.46	0.00	4.30

*** P< 0.001; ** P< 0.01; * P< 0.05



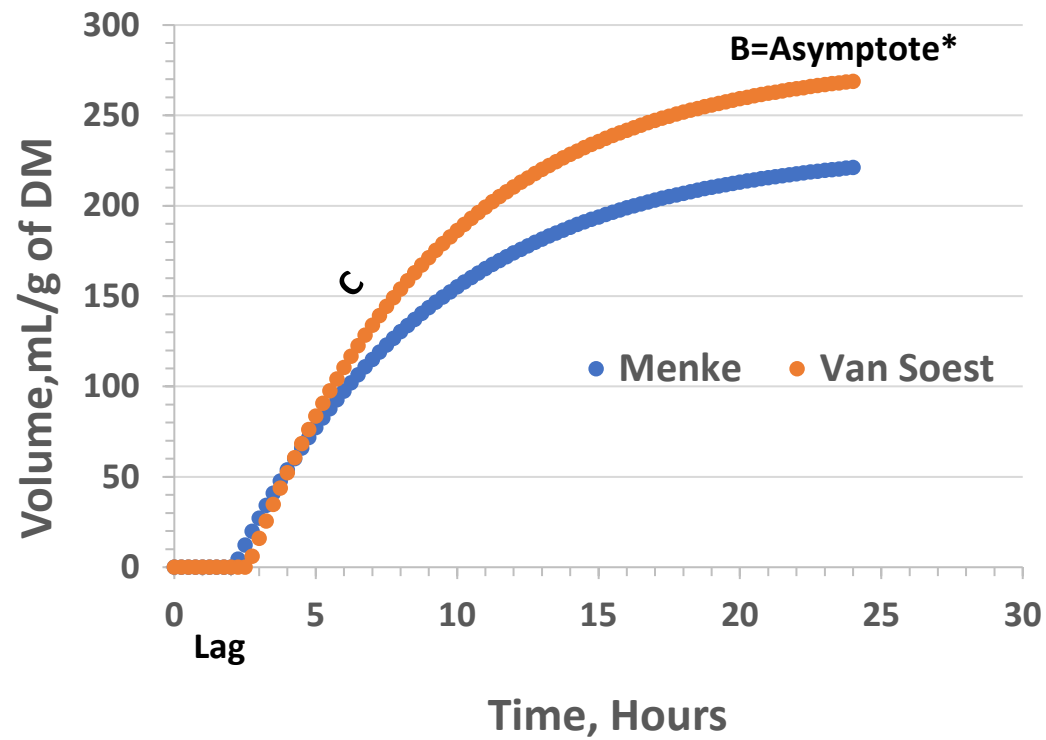
Effect of Physiological Stage



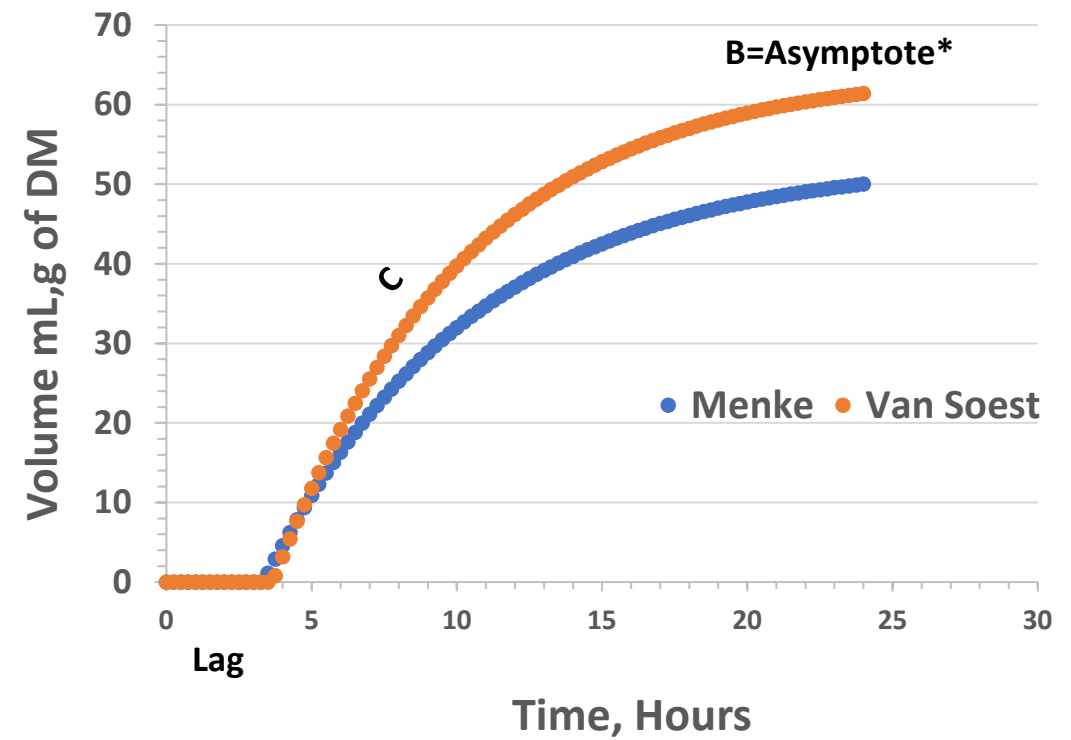


Effect of Buffer

Gas Production



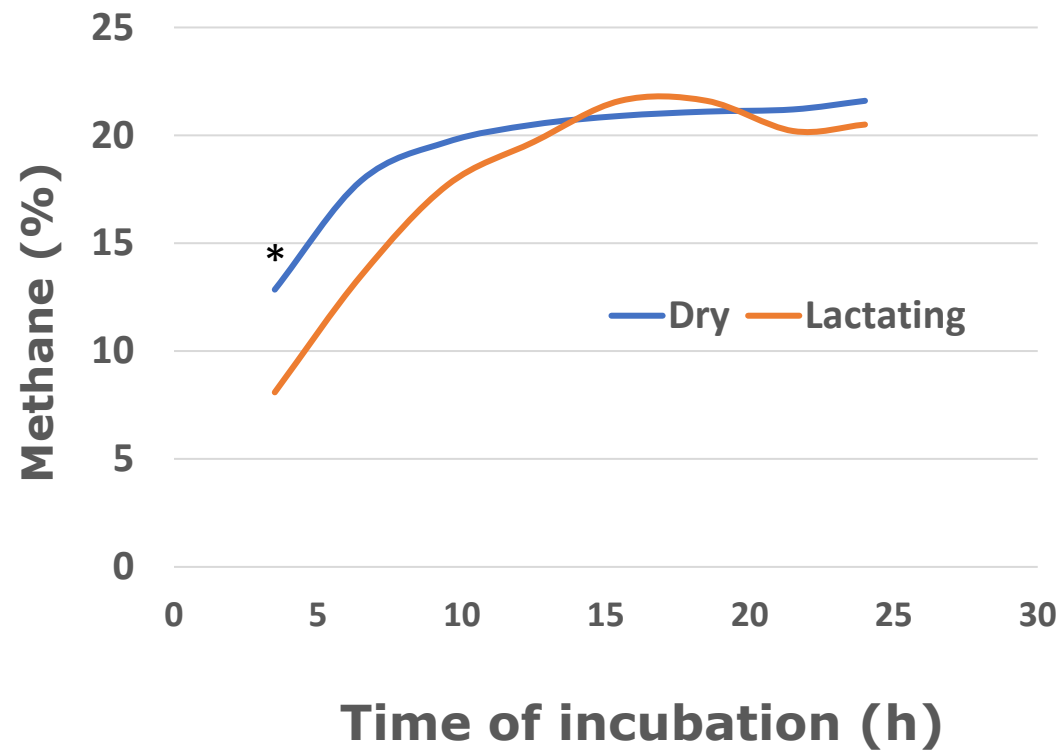
Methane Production



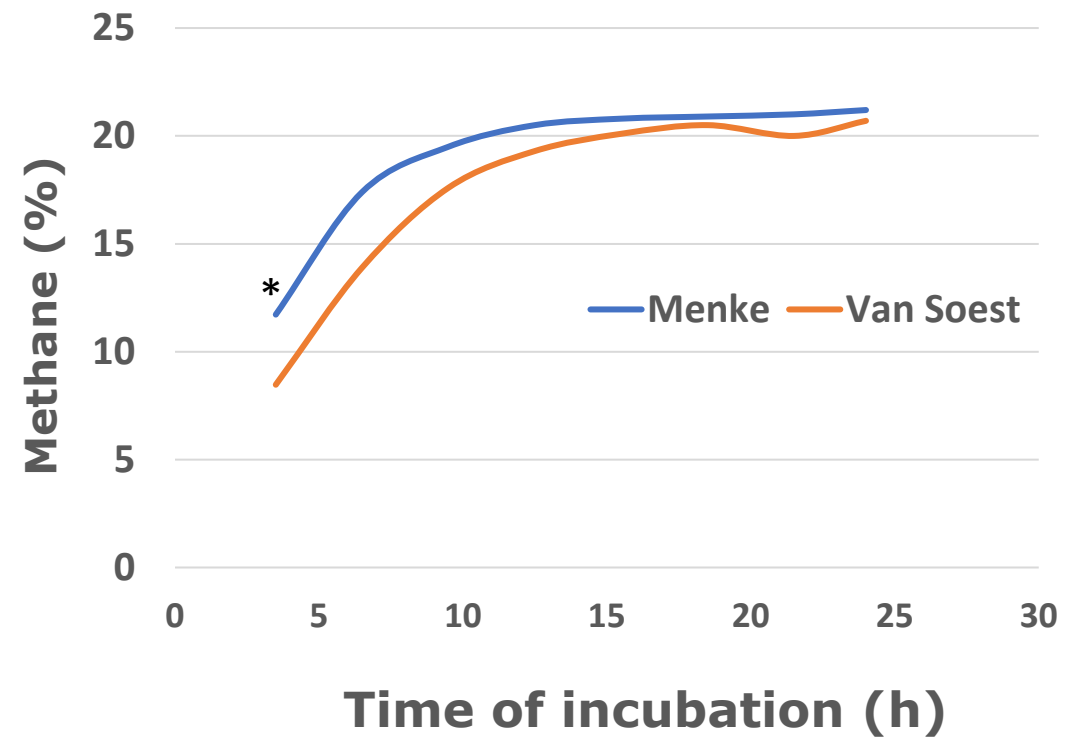


Methane (% of GP)

Effect of Physiological Stage



Effect of Buffer





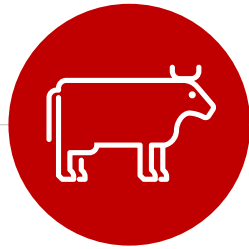
Conclusion

- The Gas Endeavour system has been effectively optimized for *in vitro* rumen fermentation studies, ensuring reliable and consistent results
- Physiological stage/feeding of cows significantly impacts cumulative gas production and methane output
- Buffer system is also a critical factor influencing gas and methane production



Acknowledgement

- This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN000000022).



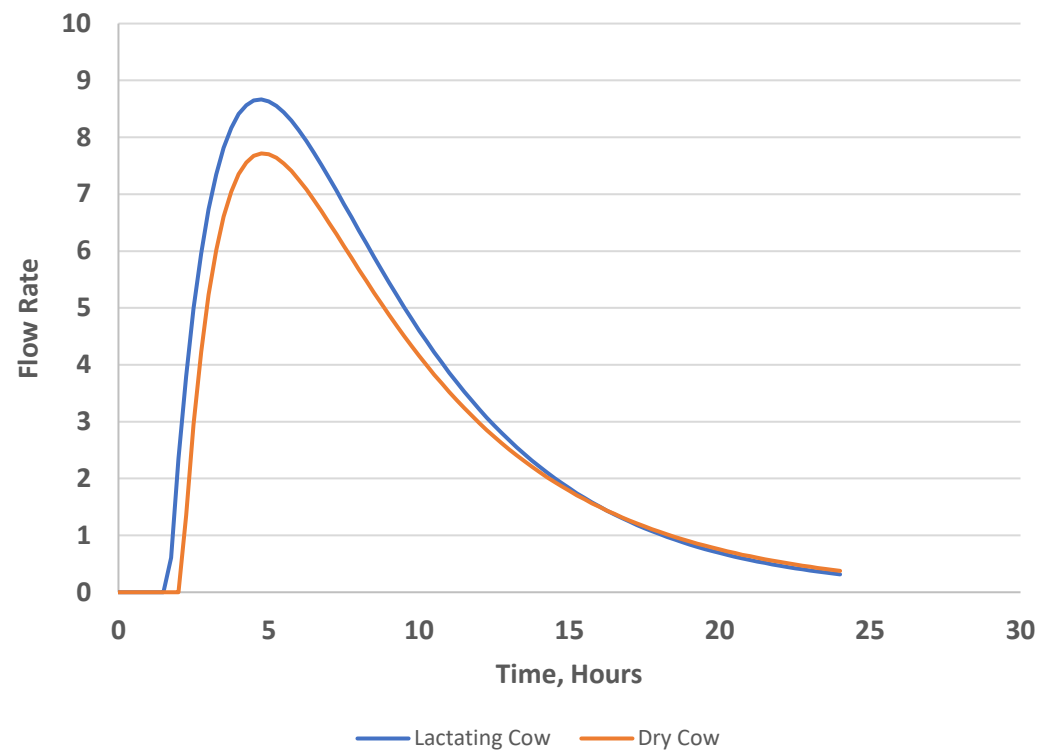
Thanks!

Any questions?

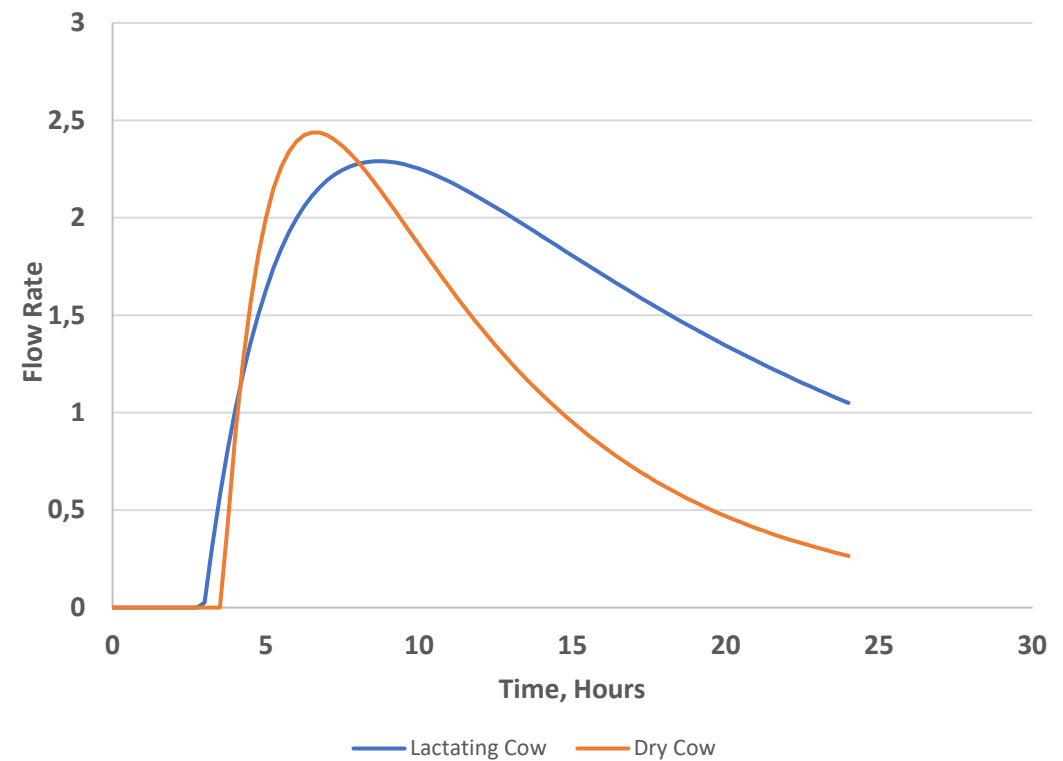


Flow Rate

Gas Production



Methane Production





Volatile Fatty Acids

Cow	Acetic acid%	Propionic acid%	iso-butyric acid %	n-butyric acid %	iso-valeric acid %	n-valeric acid%	Caproic acid%	TOT_VFA	VFA_branches
D	56.3***	21.7	1.52	12.8	3.2	2.64	1.89	3.38	7.36
L	51.3	21.6	1.67	15.4	4.14**	3.33**	2.57*	4.93**	9.14**
M	56.4	24	1.1***	13	2.37	1.71	1.46	3.53	5.19
V	51.2	19.3	2.09	15.2	4.97***	4.25***	3.00***	4.36*	11.32***