UNIVERSITÀ DEGLI STUDI DI MILANO

REE RUMEN laboratory:

Precision feeding for SARA management using essentials oils

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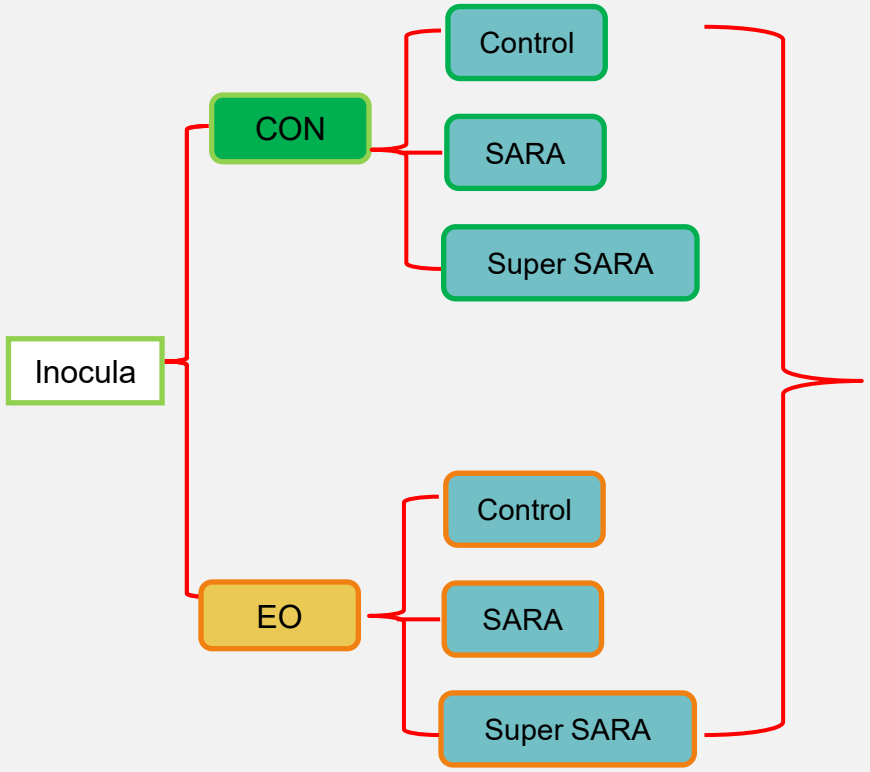
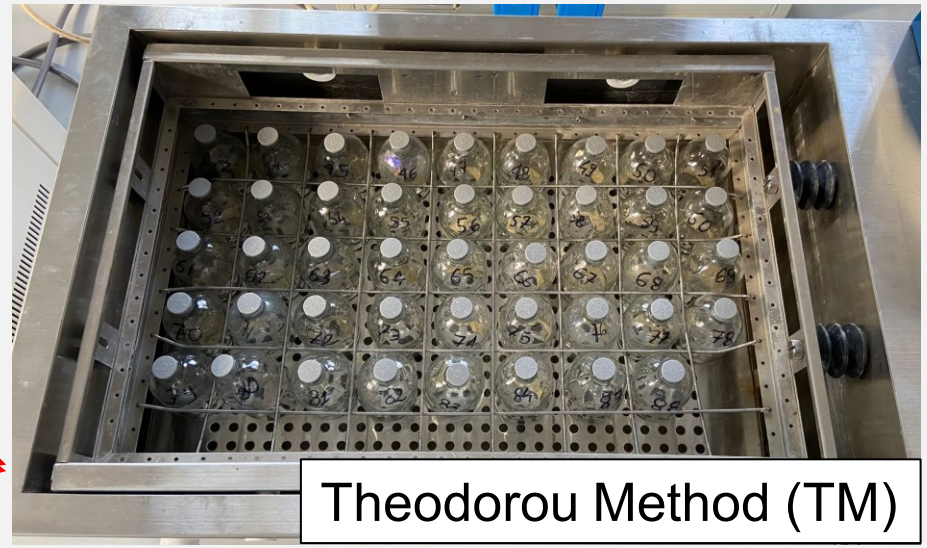
Summary

SubAcute Ruminal Acidosis < 5.8 5-6h

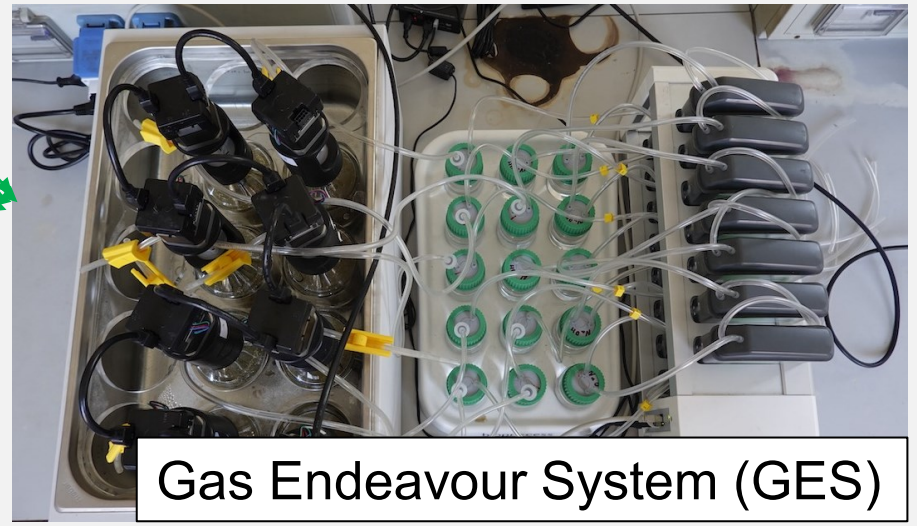
< 5.6 3h



- pH
- Gas production
- NH3
- CH4
- VFA



Theodorou Method (TM)



Gas Endeavour System (GES)

- SARA = 65% CON + 35% Wheat starch
- Super SARA = 55% CON + 45% Wheat starch

SubAcute Ruminal Acidosis

SARA is the most important nutritional disease in beef and dairy cattle and is characterized by episodes of low ruminal pH:

1. **Below 5.6 for at least 3 h/d** (Gozho et al., 2005)
2. **Below 5.8 for more than 5-6 h/d**(Zebeli et al., 2008)

The main triggers of SARA include:

1) Improper diet formulation: starch and NDF (> 3mm)

2) Non constant distribution and pourly TMR (total mixed ration)

Impacts of SARA in dairy cattle

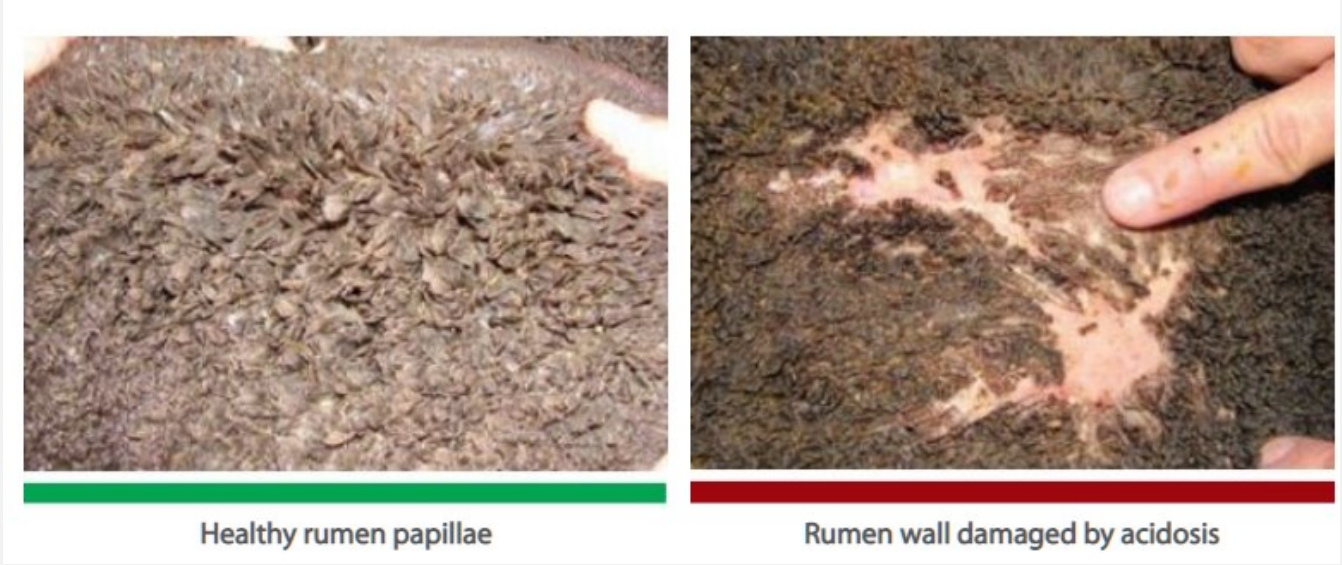
SARA leads to:

1) Reduced ingestion, rumination, salivation, milk production and fat in milk
   → < cellulolytics bacteria → < acetate

2) Diarrhea

3) Lameness

4) Infections:



→ Accelerate replacement rate → €

Precision feeding for SARA management

A) Providing correct diets: quantity, quality and mixing

B) Ensuring **continuous ration intake** throughout the day

C) Add buffers (carbonates) **or additives** to the ration, such as yeasts and **essential oils**

- **secondary metabolites** obtained mainly by steam distillation
- Very diverse composition and nature

Antimicrobial activity:

A) Lower molar proportions of acetate and a higher molar proportion of propionate → **Less acetate = less CH_4**

B) Reduce rumen protozoa and fiber degradability → **Decreasing gas production**

Goal of the study

Check whether the essential oil additive was successful in controlling the SARA problem

mitigate the lowering of pH and reduce methane production

Partial list:

- **Eugenol:** constituent of **cloves and cinnamon oils**
- **Cinnamaldehyde →** constituent of **cinnamon oils**
- **Allyl-sulfide →** compound derived from **garlic**
- **Limonene →** compound found in the **essential oils of citrus fruits.**

Materials and Methods




3 g EO

2 Control cows

3 weeks of adaptation

2 Treatment cows

CON_Rumen fluid

EO_Rumen fluid

In vitro

Substrate for in vitro incubation

1) Control diet

2) SARA diet → 65% Control diet and 35% wheat starch

3) Super SARA diet → 55% Control diet and 45% wheat starch

Unfortunately, methods of in vitro incubation in bath for the study of SARA are not optimal methods → we modify the diets and changed the buffer ratio to 1/12 *(Menke, H.H. and Steingass, H. (1988))*

Gas Endeavour

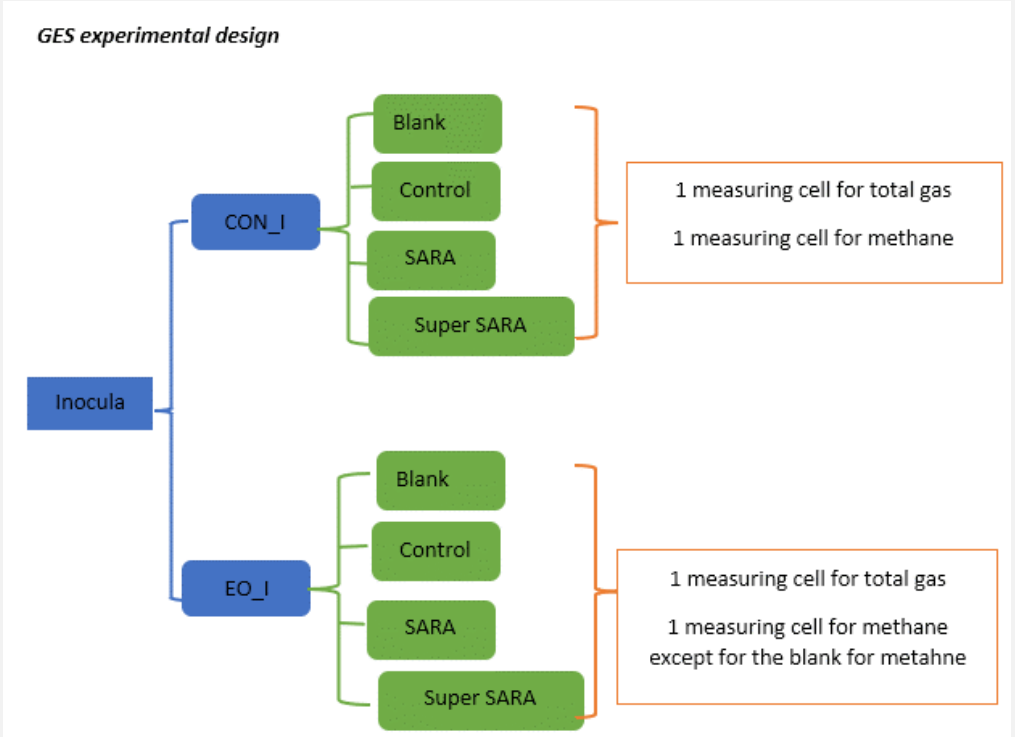
Closed, continuous gas recording system → study the kinetics of total gas and methane production.
We measured the gases produced over **24 hours**, and 8 incubation flasks and 15 measuring cells

2 days: 06/09/2022 & 08/09/2022

2 incubations

(2 inoculums * 3 diets) + blanks = 8 glass flasks

8 * 2 = 16 measuring cells (total gases and CH_4)
-1 (CH_4 cell) = 15 measuring cells



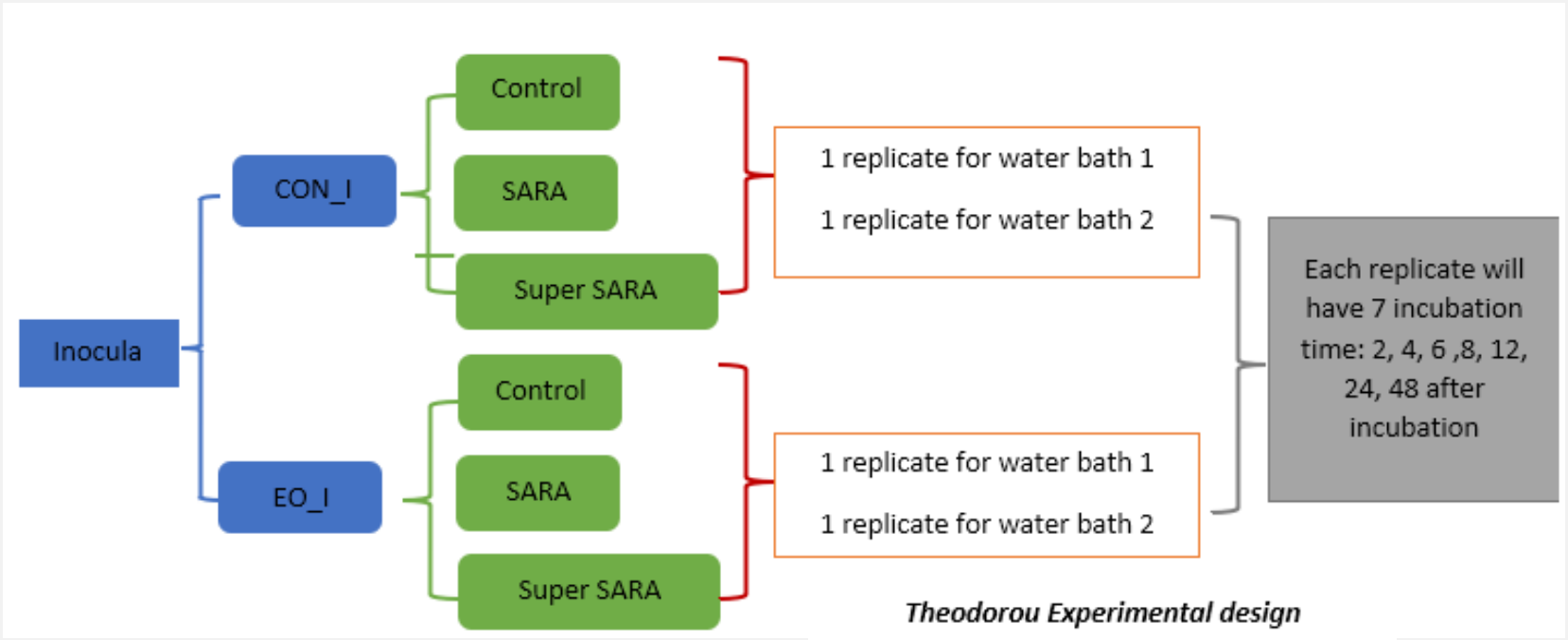
Theodorou

Theodorou method involves placing ruminal inoculum in 120-mL glass flasks in contact with the three different substrates in order to check the pressure produced and pH at different times.

It is not a continuous system !!!

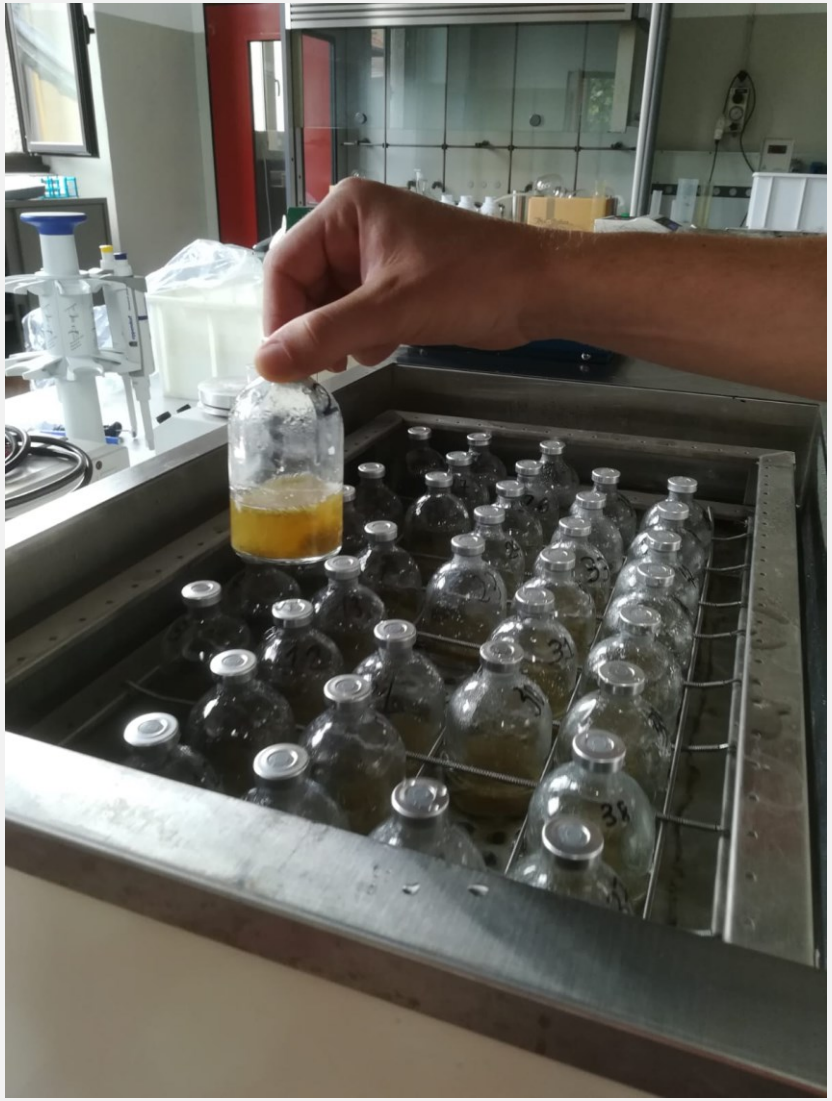
2 inoculums * 3 diets * 7 times * 2 bath = 84 vials+ 4 blanks = **88 glass vials**

1 day: 07/09/2022



Theodorou Experimental design

Theodorou



Pressure gauge

From the pressure then derive the total ml of gas produced using the **perfect gas law**

pH meter

Others analysis

AFTER GAS ENDEAVOUR:

A) First centrifugation: ammonia nitrogen and volatile fatty acids (VFA).

B) Second centrifugation: dry matter and **digestibility → a key parameter**

Statistical data analyzed using SAS software and the following gas production model:

$$Y_{ijk} = \mu + I_i + S_j + I*S_{ij} + W_k + e_{ijk}$$

Y= dependent variable

μ = general mean

I_i = inocula effect → i = 1, 2 (Control, EO)

S_j = substrate effect → j =1,..3 (Control, SARA, Super SARA)

$I*S_{ij}$ = interaction between Inocula and Substrate effects

W_k = Water bath effect → k = 1, 2

e_{ijk} = residual error

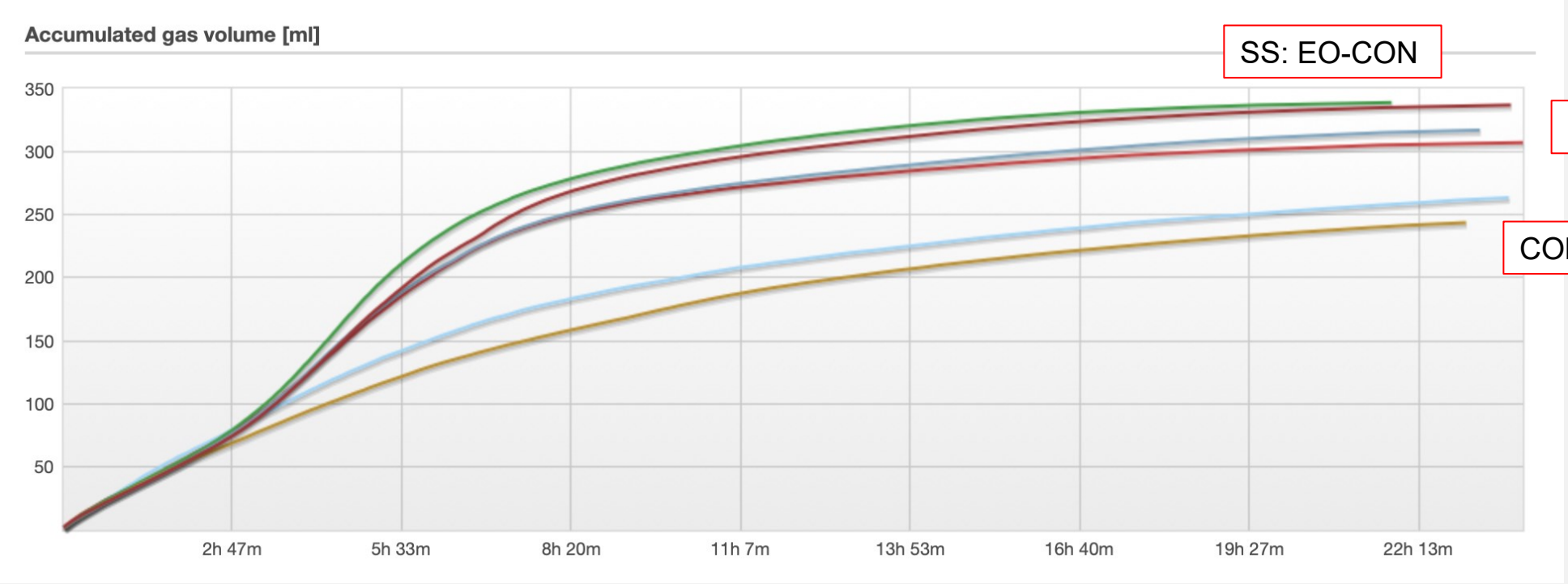
Gas Endeavour results: pH & GP

pH: inocula **tendency:** EO pH > CON pH

Inocula	pH	SE	Pvalue
CON	5.46	0.008	0.057
EO	5.49		

GP: **substrate** effect

Substrate	pH	SE	Pvalue
Control	5.73	0.01	<0.0001
SARA	5.41		
SuperSARA	5.29		

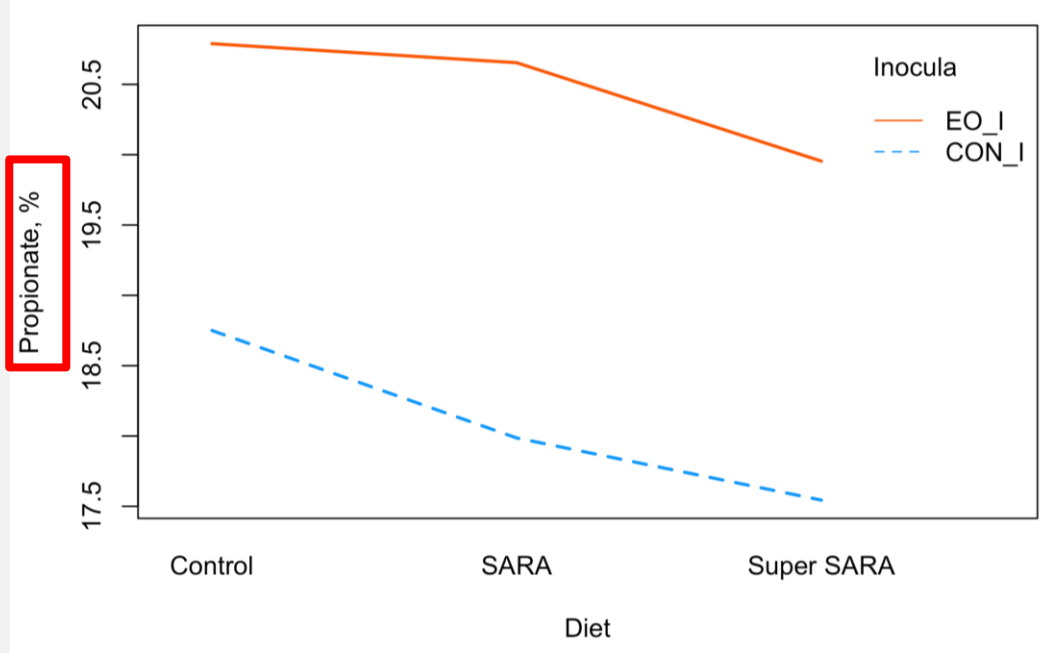


Gas Endeavour results: VFA, acetate & propionate

Total VFAs and acetic acid are not affected by **inocula**

Inocula	Total VFA mmol/l	SE	Pvalue
CON	53.6	1.006	0.37
EO	52.20		

Inocula	Acetate % TVFA	SE	Pvalue
CON	62.61	0.22	0.272
EO	62.23		



More propionate is produced with by essential oils (P = 0,0017) → amylolytic bacteria are selected

Inocula	Propionate % TVFA	SE	Pvalue
CON	18.09	0.27	0.0017
EO	20.47		

Gas Endeavour results: butyrate & NH_3

- **Less butyrate** is produced with EO (P = 0,0003)

Inocula	Butyrate % TVFA	SE	Pvalue
CON	14.45	0.12	0.0003
EO	12,94		

NH_3: inocula, substrate and run meaningfulness (P = 0.0157, P = 0.0031 and P = 0,0157) : NH_3 with EO is higher → **why?**

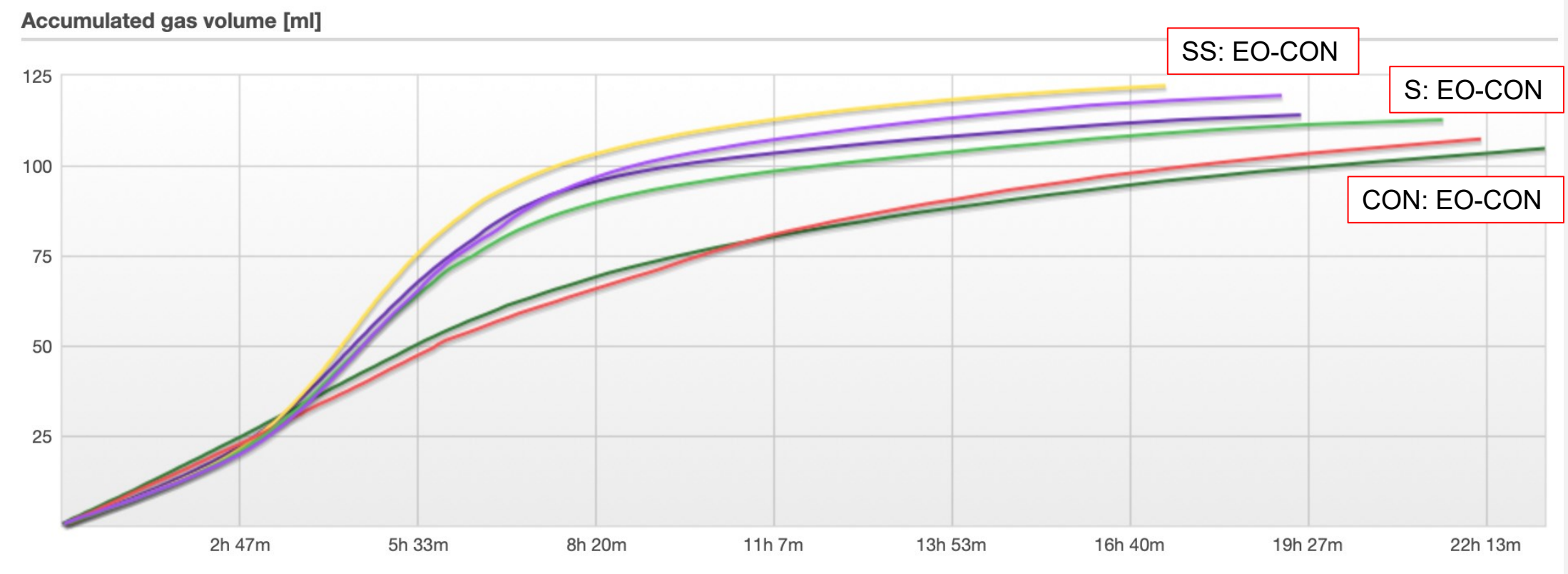
NH_3 is an important growth factor for the main butyrate-producing bacterium

Inocula	NH3 mgl/l	SE	Pvalue
CON	0.13	0.007	0.0157
EO	0.16		

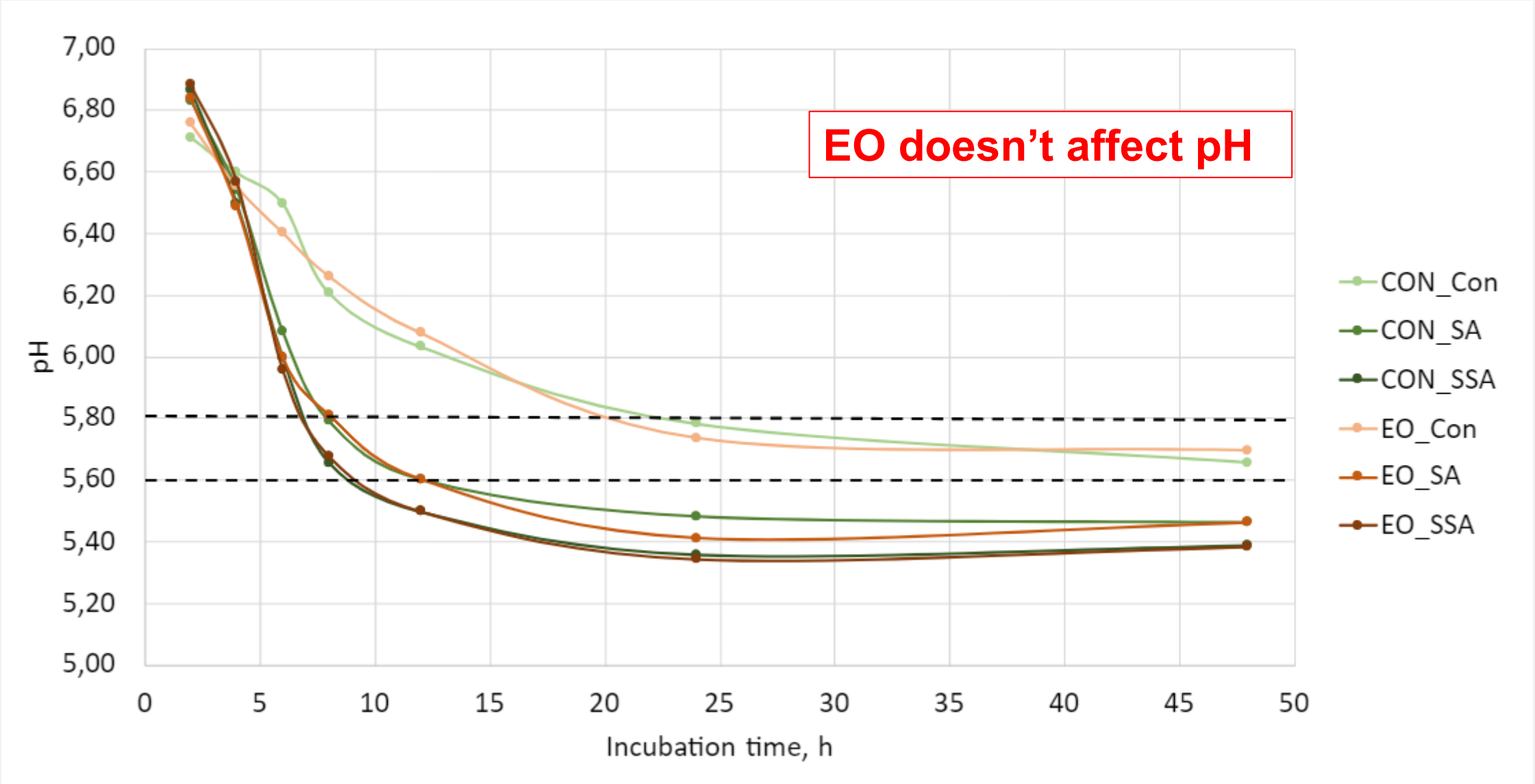
Gas Endeavour results: CH_4

Methane production is NOT affected by essential oils as we would expect

Inocula	CH4 ml/g DM	SE	Pvalue
CON	26.58	0.55	0.77
EO	26.33		

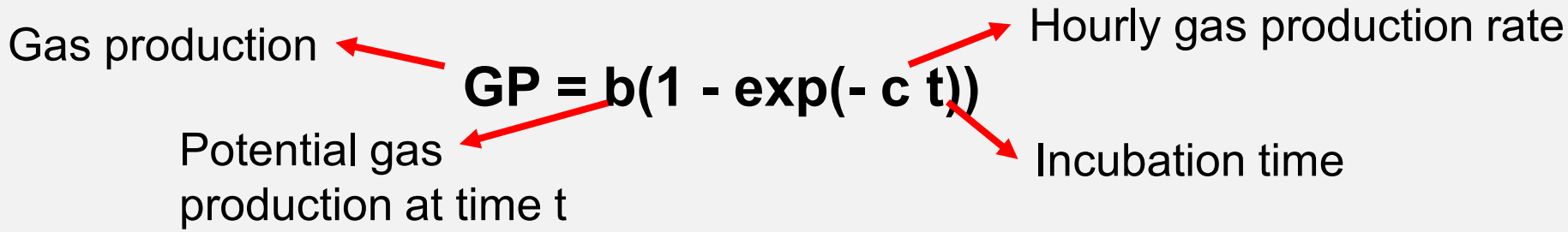


Theodorou results: pH



Theodorou results: potential gas production

Exponential model without latency phase



Potential gas production 48h: only inocula meaningfulness

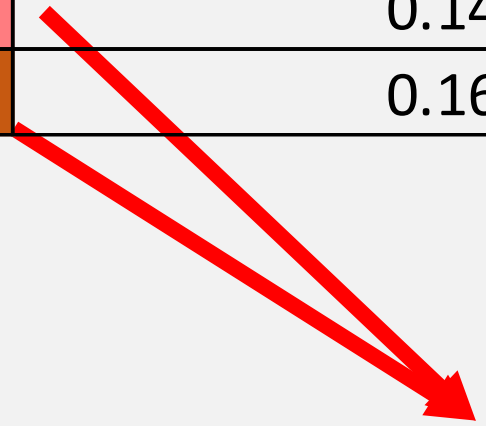
Inocula	Potenzial gas produciton ml	SE	Pvalue
CON	111.7	1	0.004
EO	105.5		

Microbic selection → less cellulolytic bacteria?

Theodorou results: hourly gas production

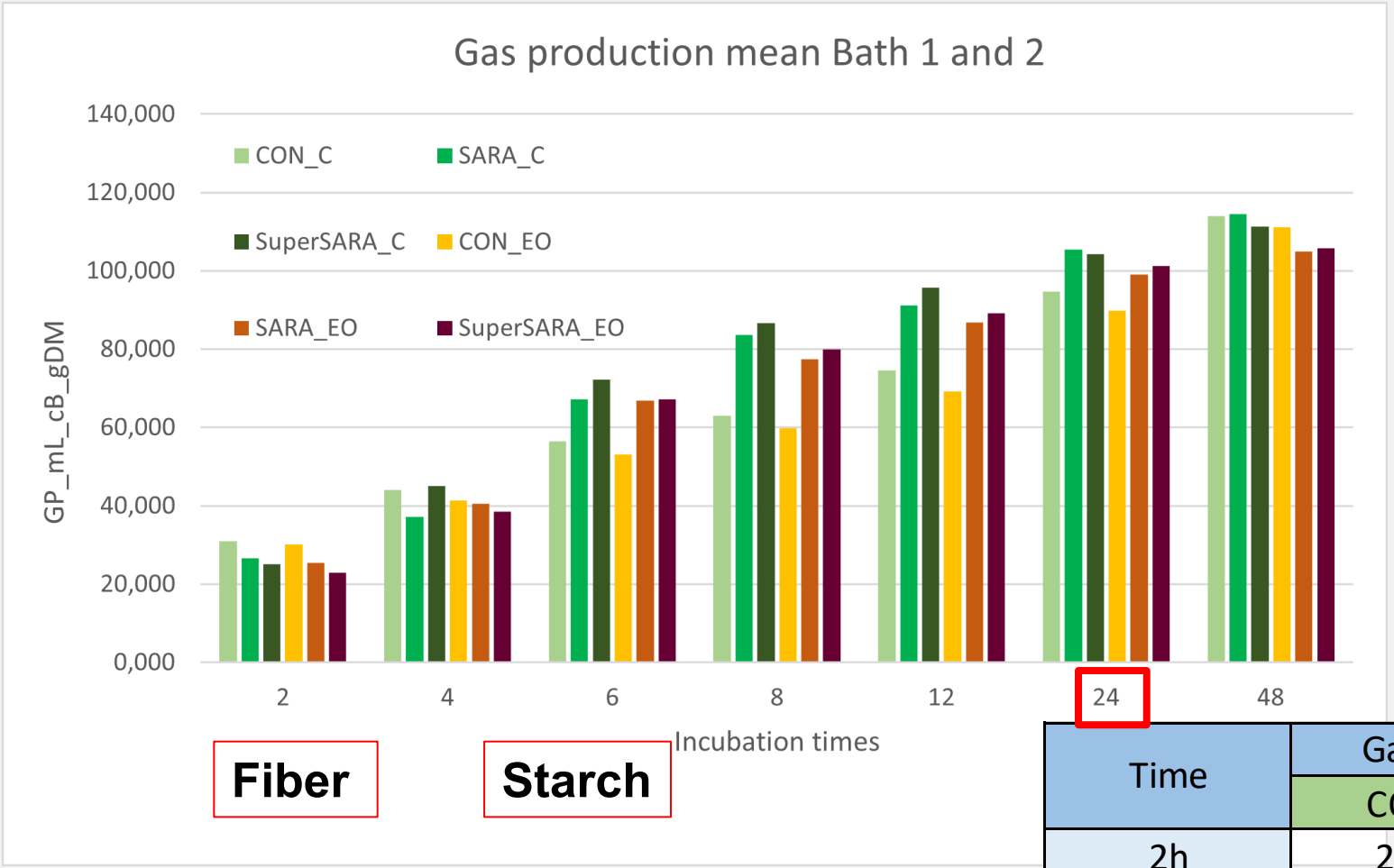
Hourly gas production rate 48h: interaction and substrate meaningfulness

Substrate	Hourly gas production rate	SE	Pvalue
Control	0.11	0.003	<0.0001
SARA	0.14		
SuperSARA	0.16		



> Starch

Theodorou results: Gas production



Essetials Oils produces LESS gas

Time	Gas production ml		SE	Pvalue
	CON_I	EO_I		
2h	27.53	26.16	0.84	0.297
6h	65.3	62.35	2.13	0.365
12h	87.15	81.75	1.92	0.094
24h	101.47	96.68	1.32	0.043
48h	113.27	107.29	1.8	0.057

Conclusions:

A) Incubation tecnique

1) Gas Endeavour → tendency for EO to slightly raise pH, increase NH_3, increase propionate and reduce butyrate. No effects on CH_4 production and GP.
2) Theodorou → NO significant difference in pH but essential oils lead to lower total gas production

B) Additive:

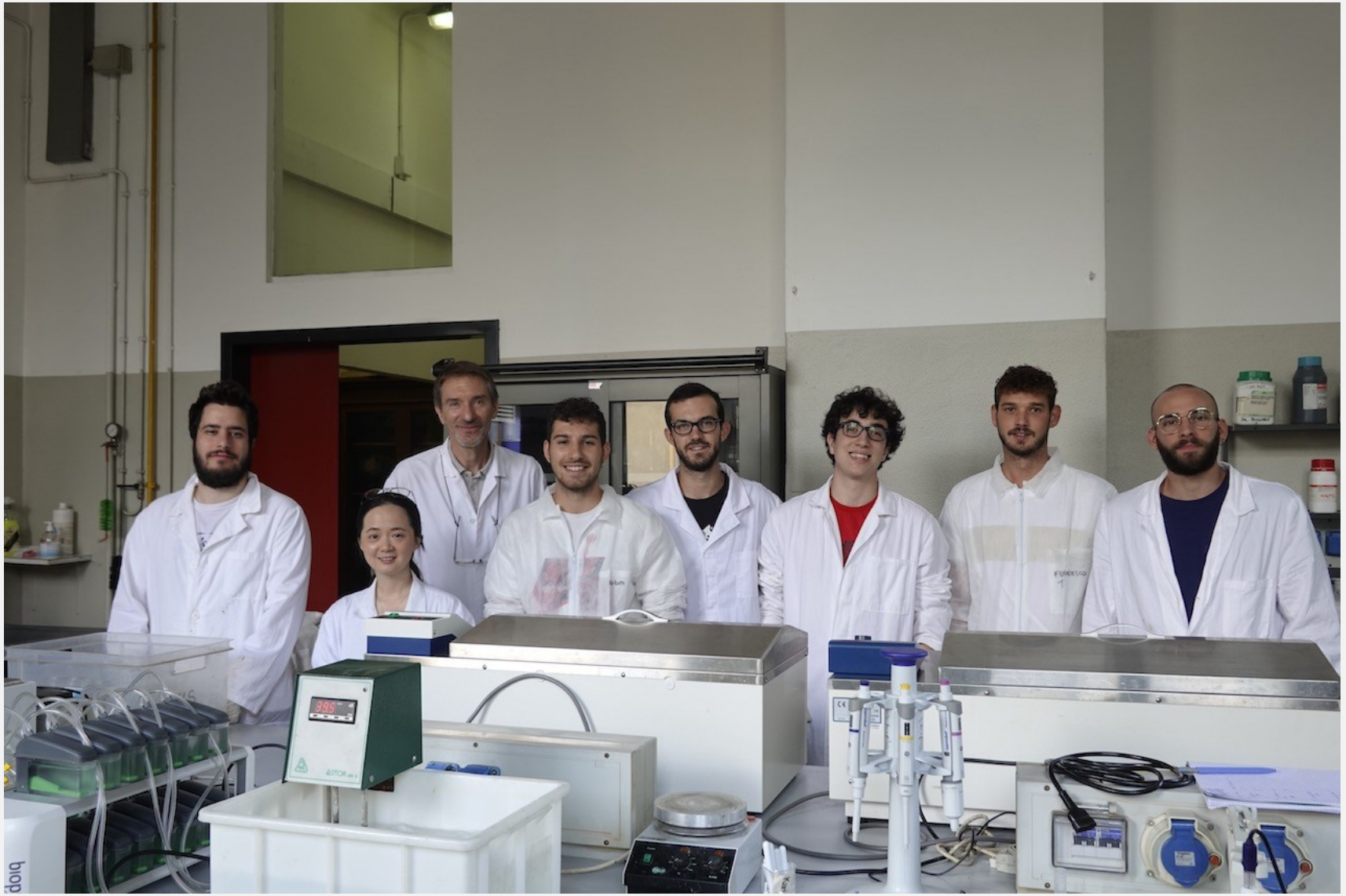
1) Essential oils could influence methanogenesis, **but** this was not observed with our methods
2) No digestibility effect but less palatability → molasses

Inocula	DMD	SE	Pvalue
CON	1.37	0.02	0.76
EO	1,26		

C) Procedure

This trials are part of a larger experiment where other aspects such as lactic acid, lipopolysaccharide, and bacterial populations are evaluated → **TIME**

Thanks for the attention



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