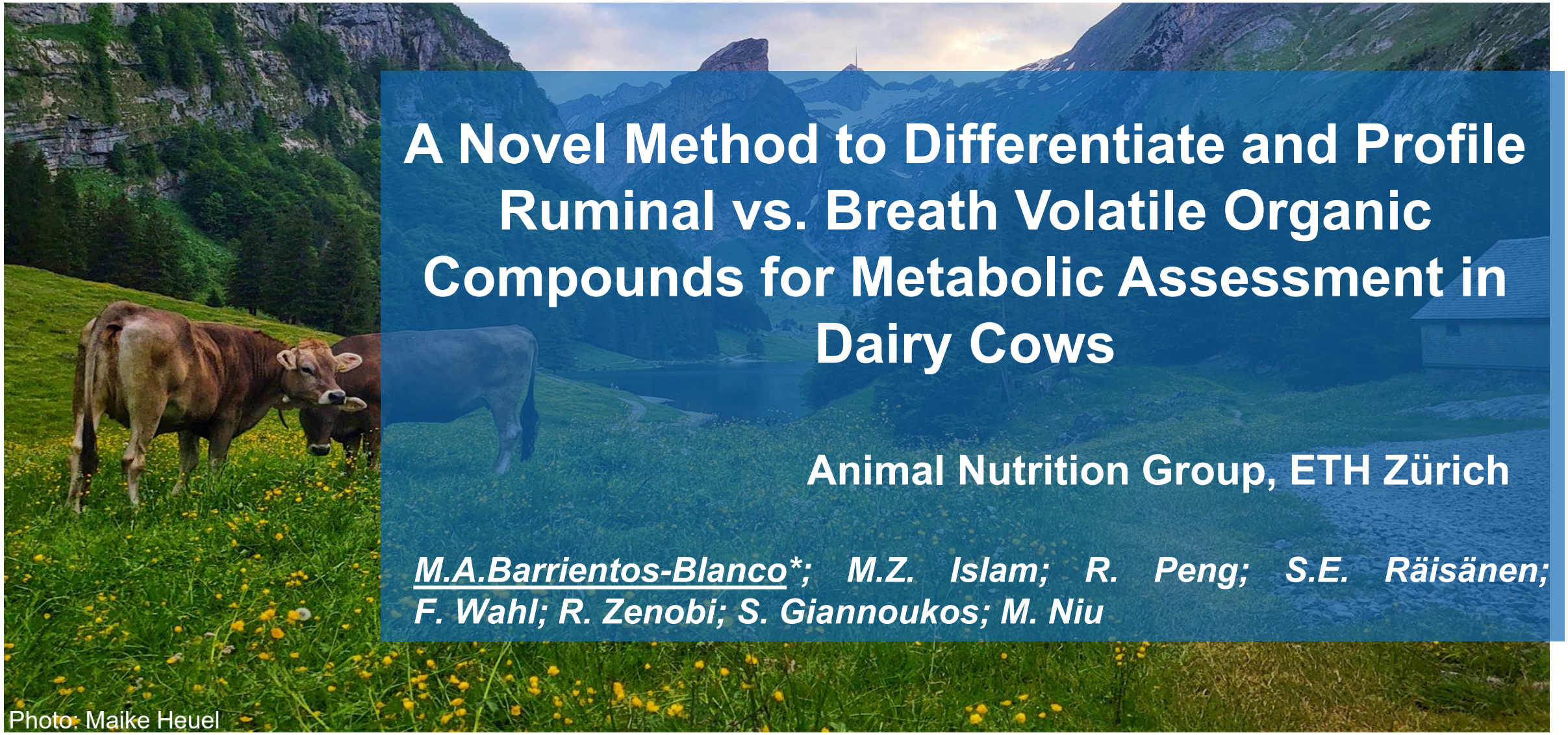
ETH zürich



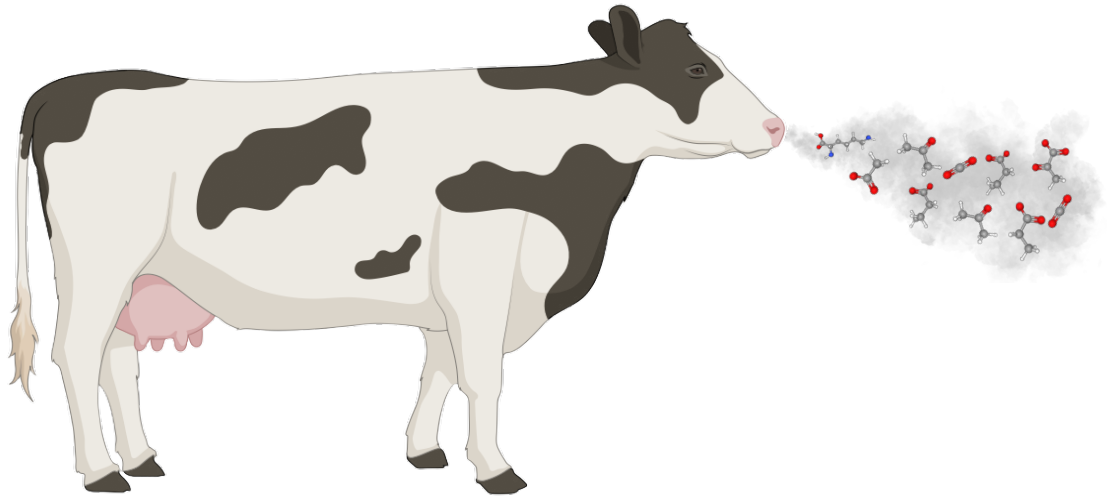
A Novel Method to Differentiate and Profile Ruminal vs. Breath Volatile Organic Compounds for Metabolic Assessment in Dairy Cows

Animal Nutrition Group, ETH Zürich

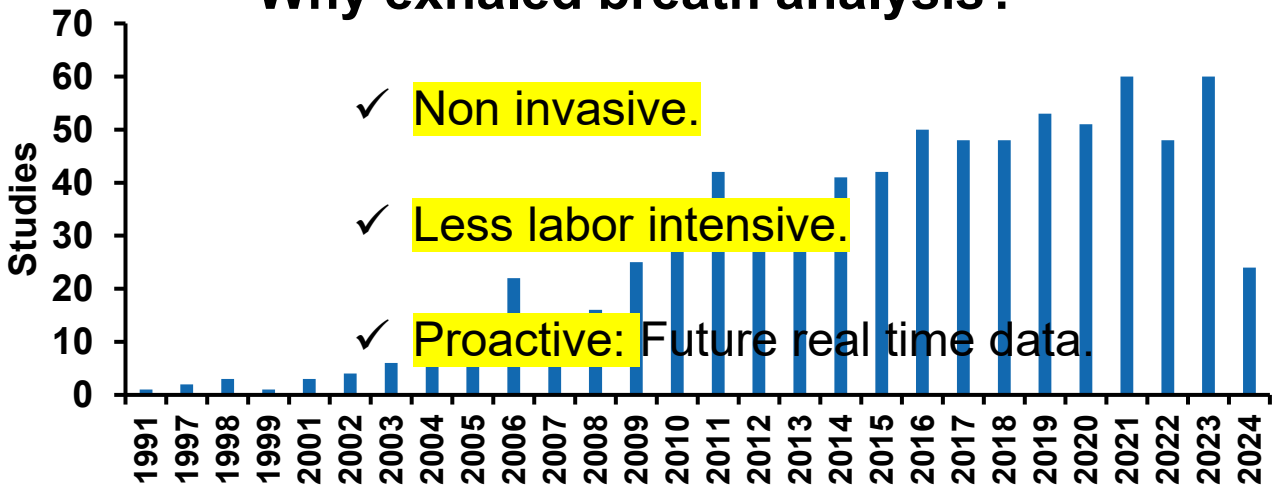
***M.A.Barrientos-Blanco**; M.Z. Islam; R. Peng; S.E. Räisänen; F. Wahl; R. Zenobi; S. Giannoukos; M. Niu*

Photo: Maike Heuel

Exhaled Breath Analysis



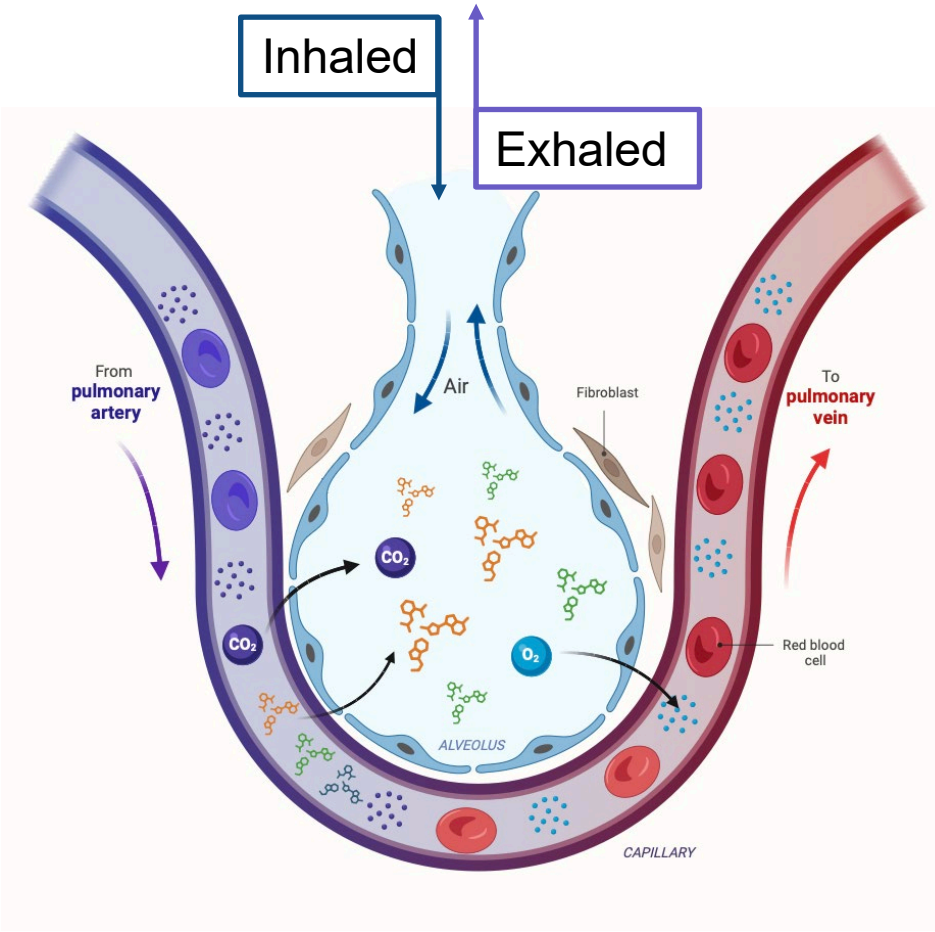
Why exhaled breath analysis?



- ✓ Non invasive.
- ✓ Less labor intensive.
- ✓ Proactive: Future real time data.

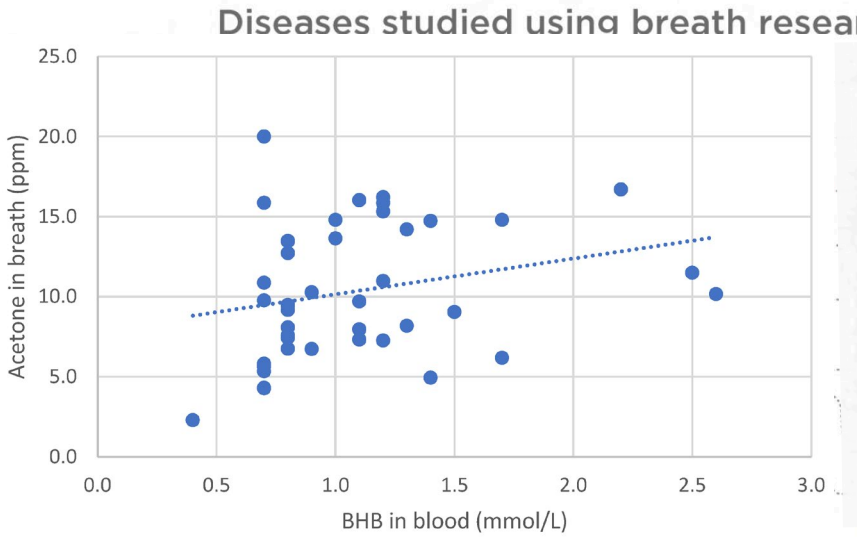
Clinicaltrials.gov., 2024

VOCs: Volatile Organic Compouds

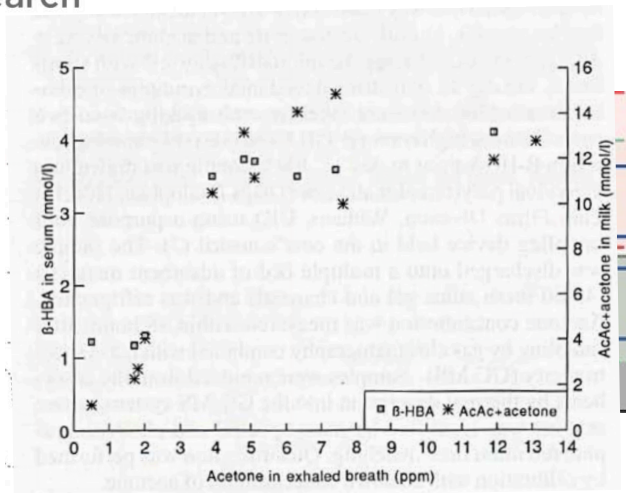


Zurich Exhalomics

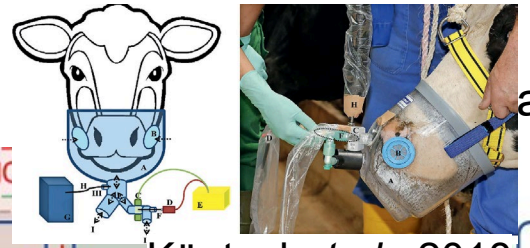
Exhaled Breath Analysis



van Erp der Kooij et.al., 2023

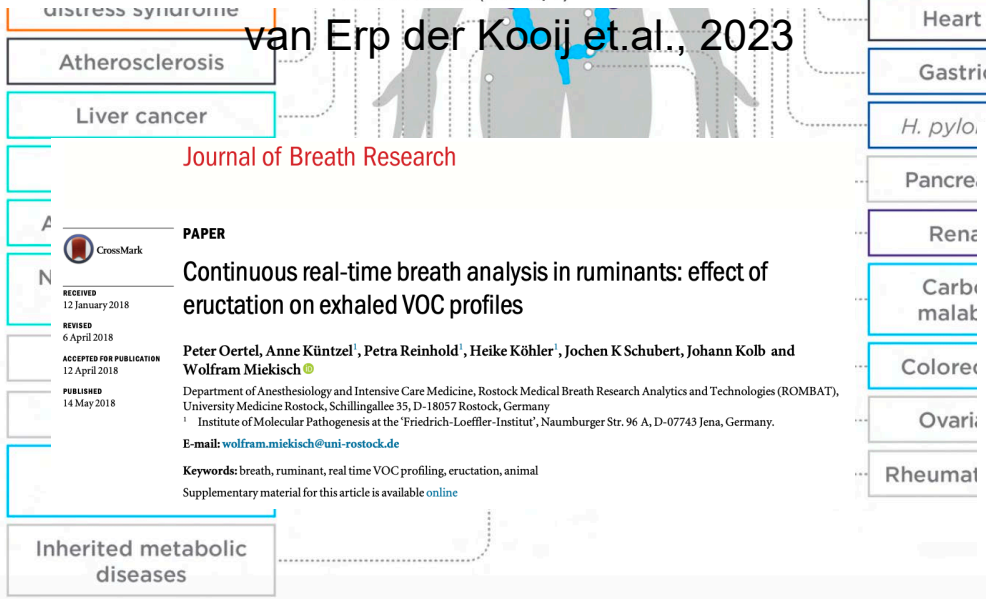


Dobbelaar et.al., 1996



al., 2024.

Küntzel *et.al.*, 2018



Journal of Breath Research

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Continuous real-time breath analysis in ruminants: effect of eructation on exhaled VOC profiles

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Keywords: breath, ruminant, real time VOC profiling, eructation, animal

Supplementary material for this article is available online

Molecular and Cellular Biochemistry (2023) 478:2473–2480
https://doi.org/10.1007/s11010-023-04676-5

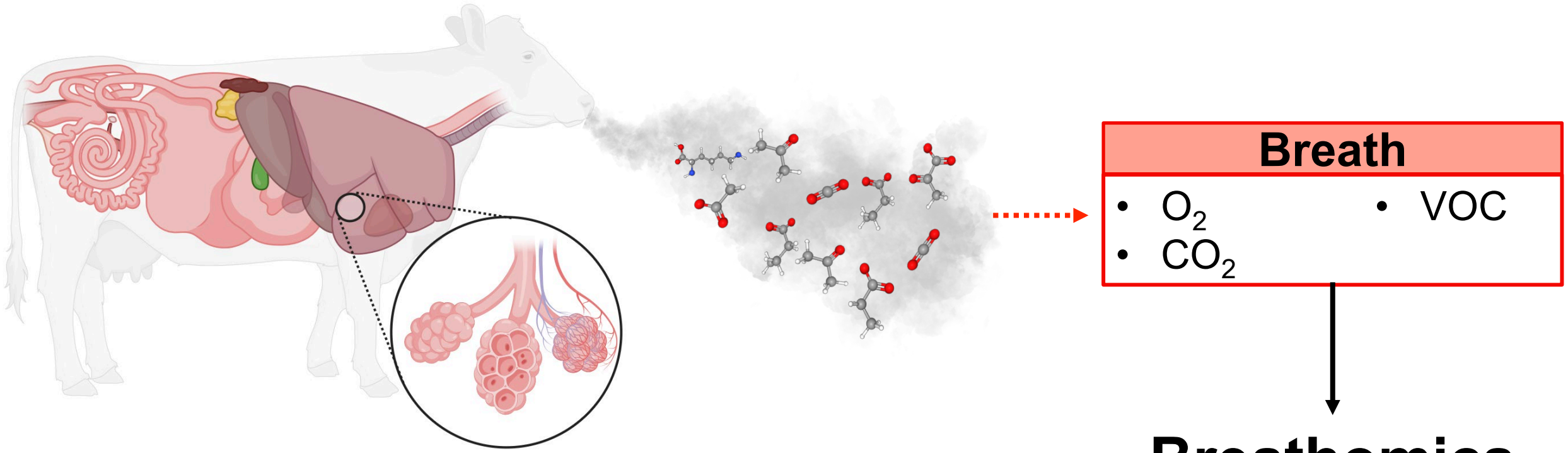
Check for updates

Putative volatile biomarkers of bovine tuberculosis infection in breath, skin and feces of cattle

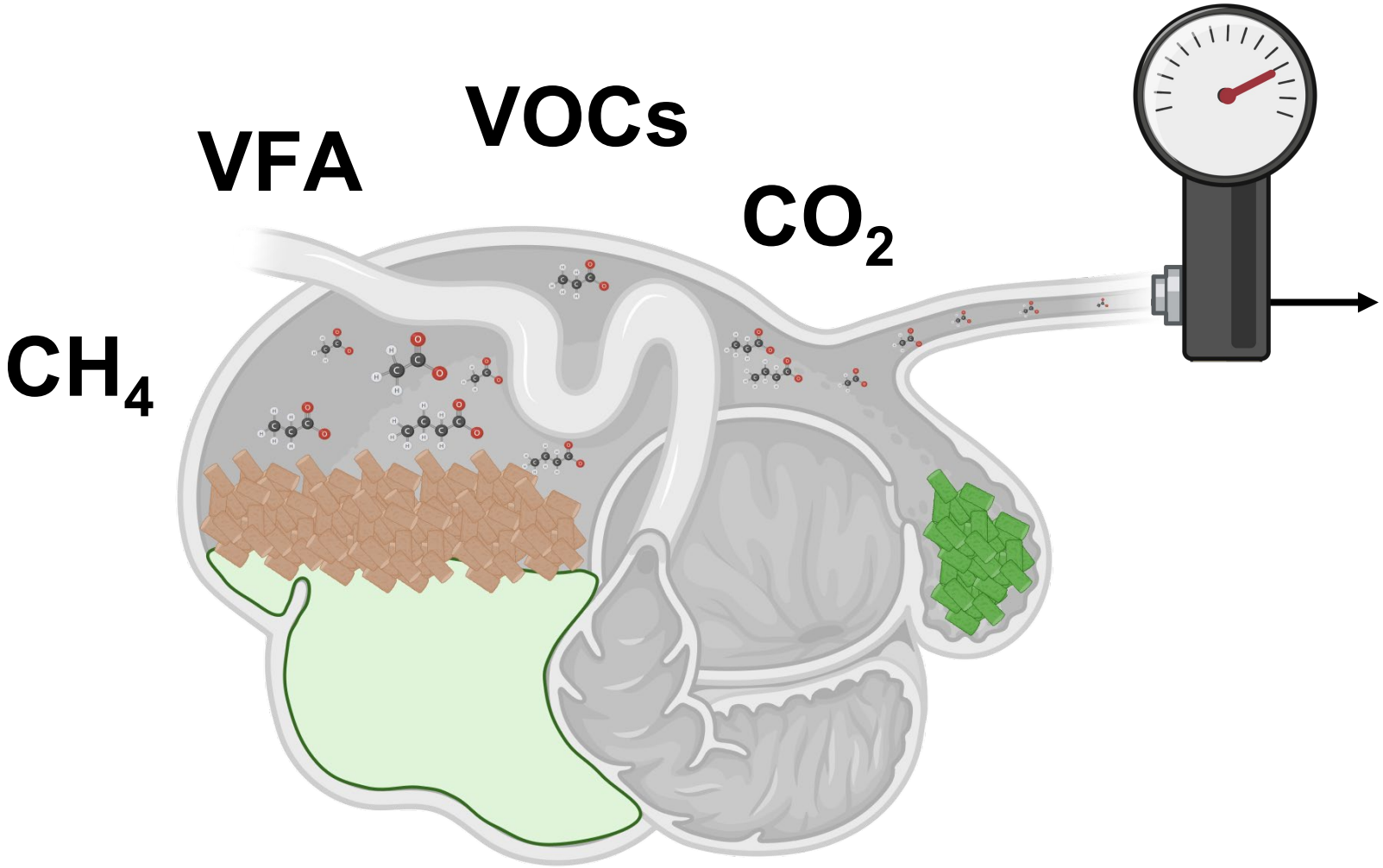
Mihai Brebu[1] · Violeta Elena Simion[2] · Viorel Andronie[2] · Aylen Lisset Jaimes-Mogollón[3] · Kelvin de Jesús Beleño-Sáenz[3,4] · Florina Ionescu[5] · Tesfalem Geremariam Welearegay[5] · Raluca Suschinel[6] · Jose Bruno de Lema[6,7] · Radu Ionescu[7]

Figure 5.11 - OMED Health Breath Analyzer for diagnosing functional gastrointestinal disorders will be available by the end of 2023.

Breath: blood borne metabolites

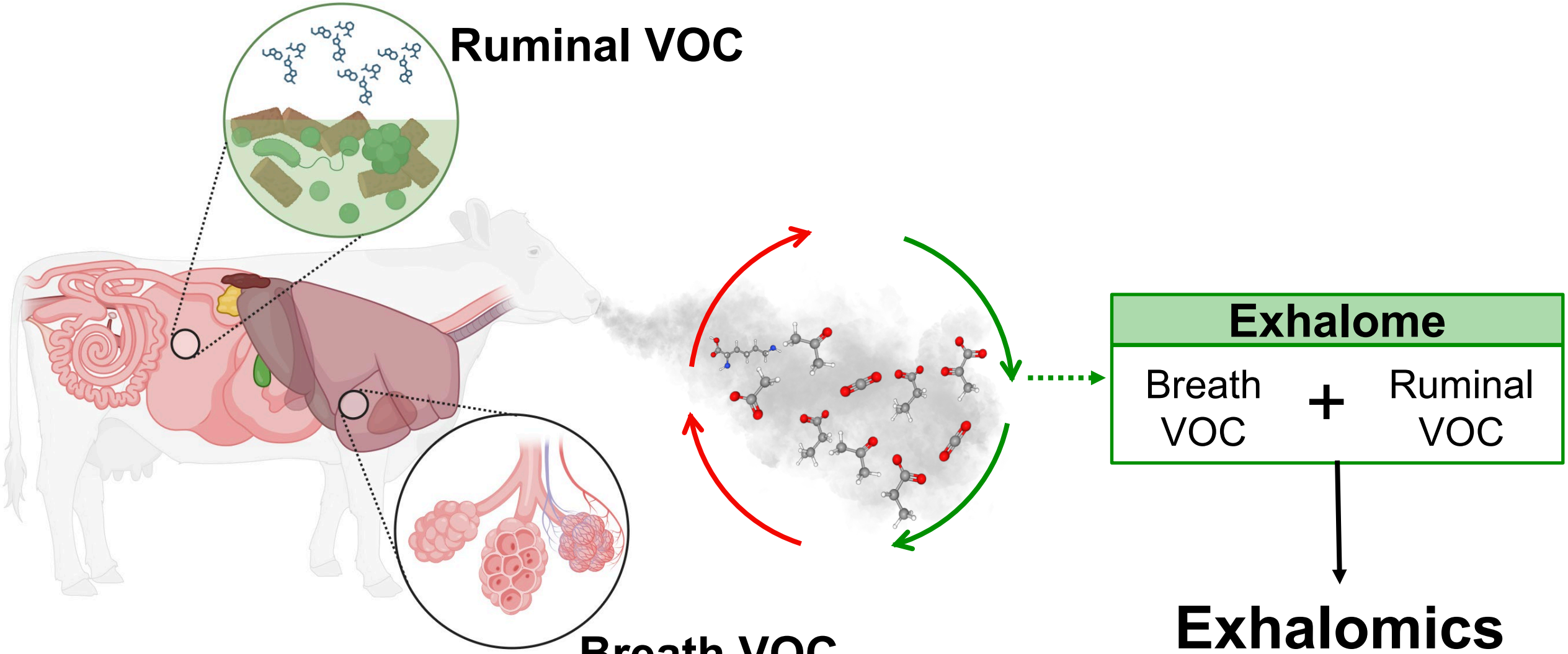


Cow's burping releases rumen preassure



Eructations releases rumen gases

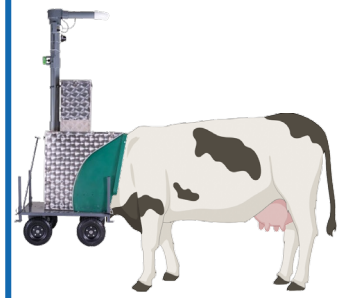
Frequent erucation mix ruminal and breath VOC

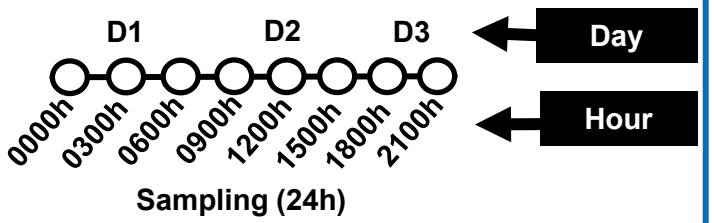


Can exhalomics be a non-invasive approach to assess rumen function?

Using exhalomics to explore rumen fermentation

Study Design





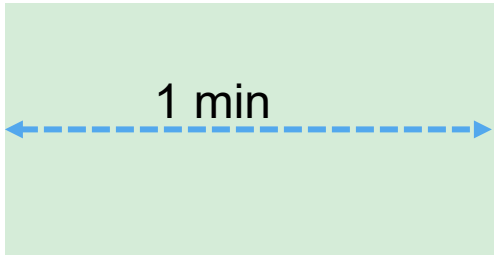
Sampling (24h)

*n = 7 dairy cows
*Basal diet
*Feeding = 1x/d

○ Secondary Electro Spray Ionization High Resolution Mass Spectrometer (SESI) analysis.

Exhalome Sampling

Sampling: 30s after eructation event, 1x.



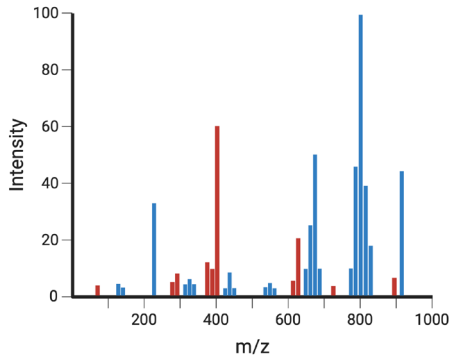
Time

(Islam et al., 2023a)

https://doi.org/10.3168/jds.2023-23301

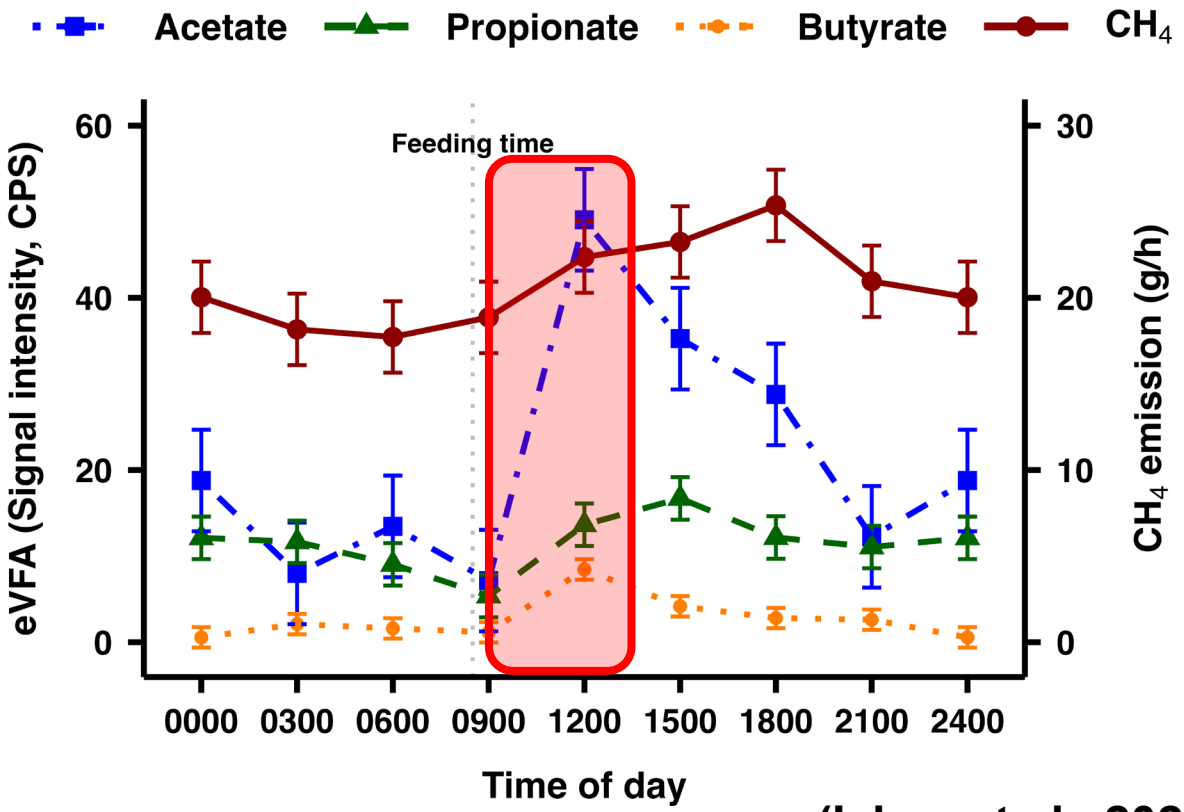
2

3



SESI-HRMS Analysis

Can exhalomics assess ruminal fermentation?



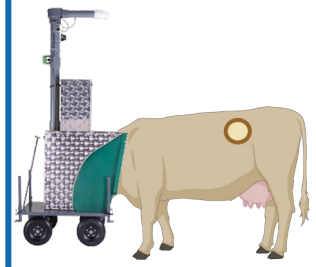
(Islam et al., 2023a)

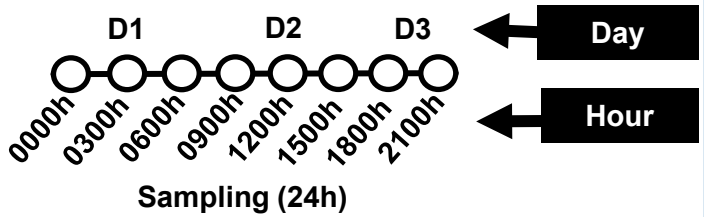
- Exhaled VFA (eVFA) increased after feeding.

Can exhalomics replace rumen sampling?

Can exhalomics replace rumen sampling?

Study Design

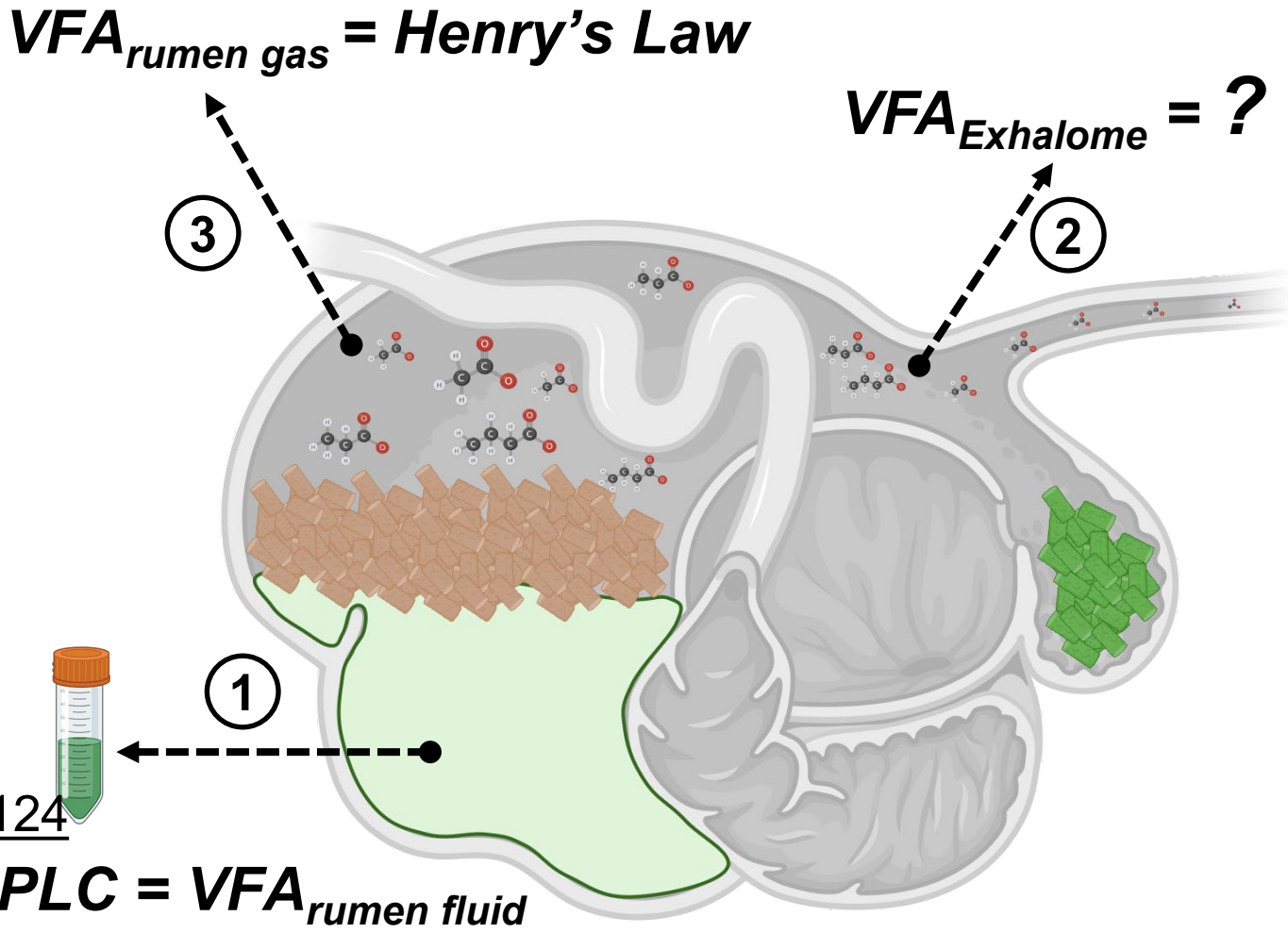




*n = 4 Original Brown Swiss
*Trt. = 16% vs. 6% of Starch
*Feeding = 1x/d.
*Switch back design

(Islam et al., 2023b)

https://doi.org/10.3168/jds.2023-24124



No interactions between VFA measurement method and diet

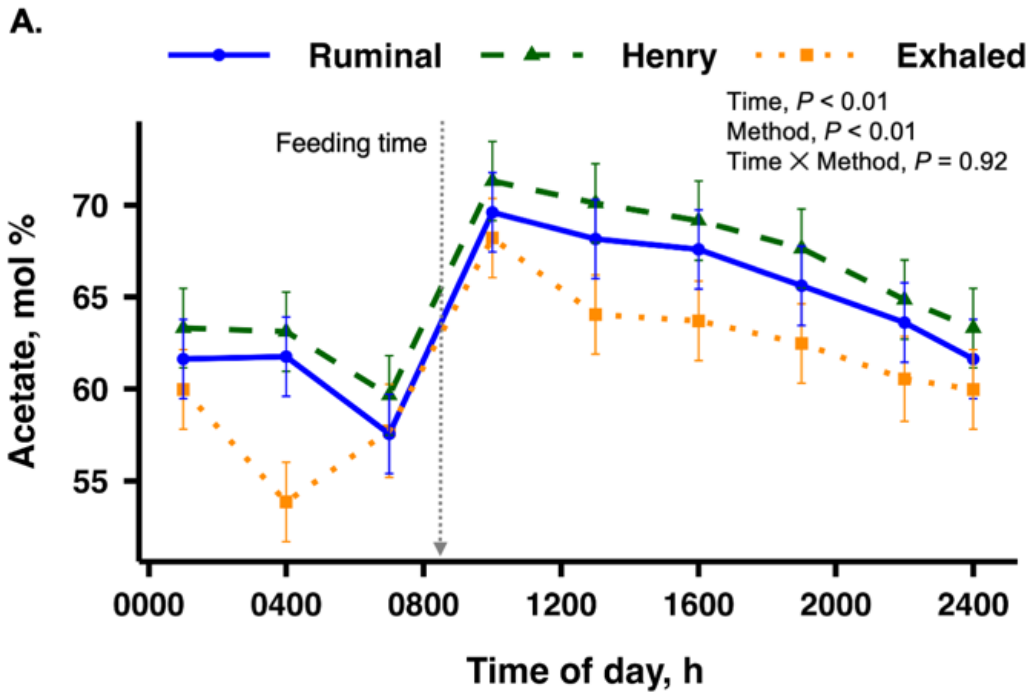
	Least Square Means							*P*-value		
	HS-R	LS-R	HS-H	LS-H	HS-E	LS-E	SE	Diet	Method	**Diet × Method**
% of total VFA										
Acetate	62.7	62.0	64.4	63.7	59.4	57.0	2.14	0.41	< 0.01	0.77
Propionate	22.2	23.8	24.4	26.1	27.1	28.0	1.44	0.19	< 0.01	0.85
Butyrate	13.2	12.2	9.54	8.49	8.51	8.82	1.014	0.51	< 0.01	0.30
A:P	3.01	2.82	2.81	2.64	2.29	2.22	0.283	0.43	< 0.01	0.87

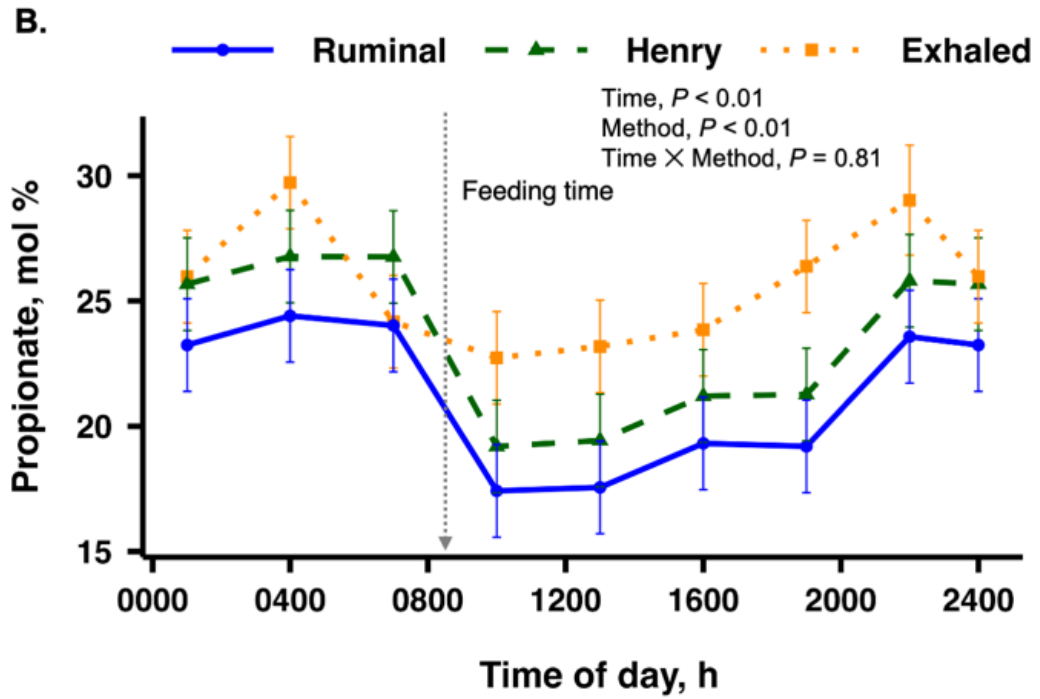
HS = High starch; LS = Low starch; E = Exhaled; R = Ruminal; H = Henry's law-corrected

(Islam et al., 2023b)

No interactions between VFA measurement method and time

High-starch diet





(Islam et al., 2023b)

Blood Borne
Volatile Metabolites

Breath

- O_2
- CO_2
- VOC

Ruminal exhaled gases may confound breath biomarkers used for metabolic assessment in dairy cows

Ruminal Volatile
Metabolites

Exhalome

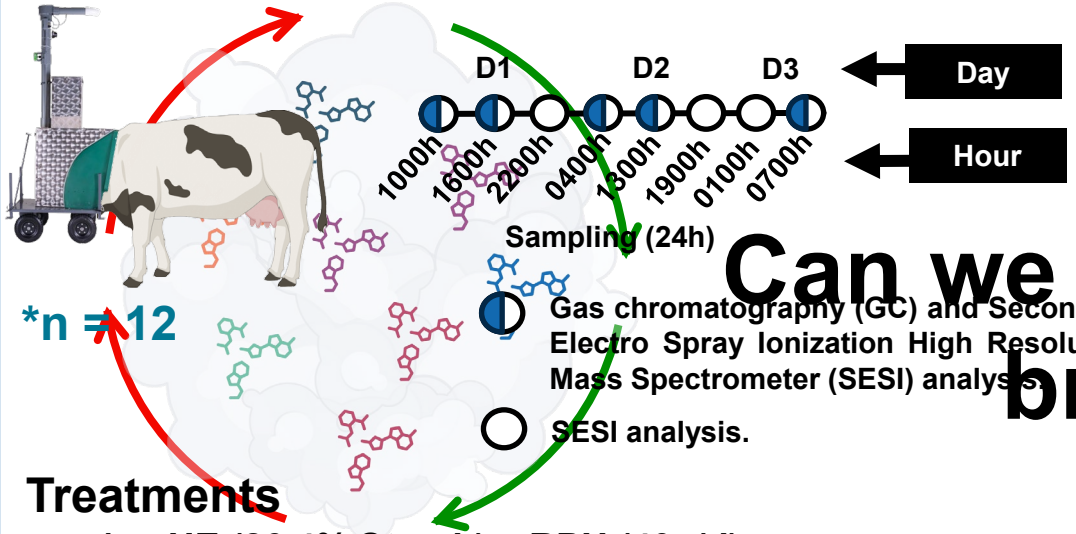
- Breath VOC
- Ruminal VOC

A new method to differentiate the origin of exhaled VOC

Breath

- O_2
- CO_2
- VOCs

4 x 4 Latin Square Design



Treatments

1. HE (26.4% Starch) + RPH (40g/d).
2. HE + CON (20g/d coating material).
3. LE (16.4% Starch) + CON
4. LE + RPH.

Exhalome

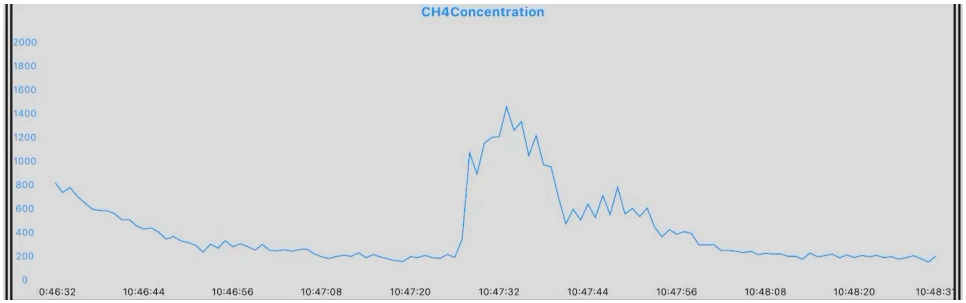
- Breath VOC
- Ruminal VOCs

Exhalome	CH_4 peak (>500 & <250 CH_4 ppm), 2x
Breath	CH_4 ~150 ppm (10s), 3x

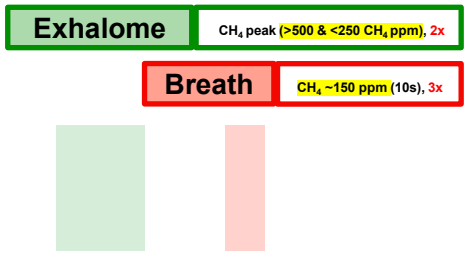
Ex Br

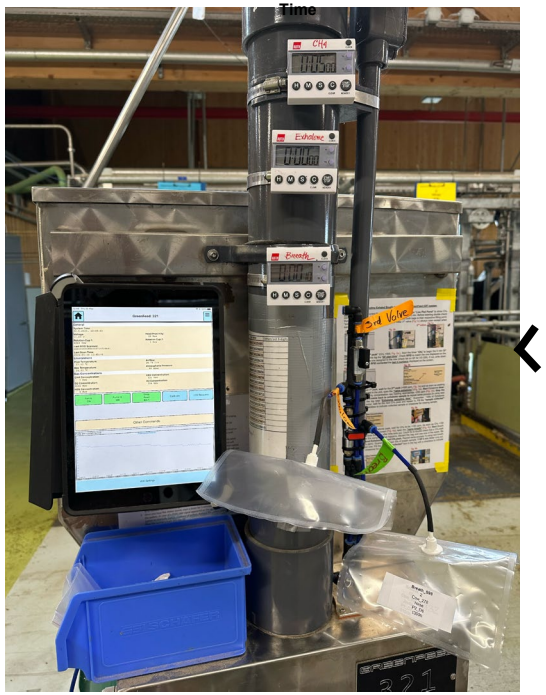
Can we differentiate ruminal from breath exhaled VOCs ?

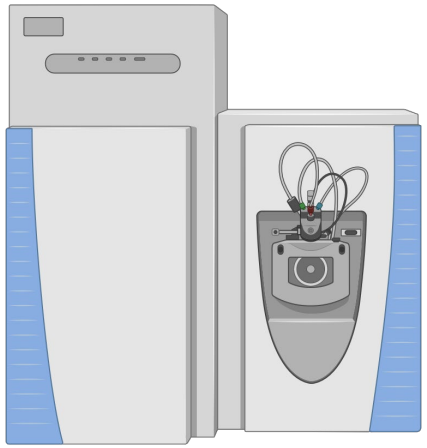
Time



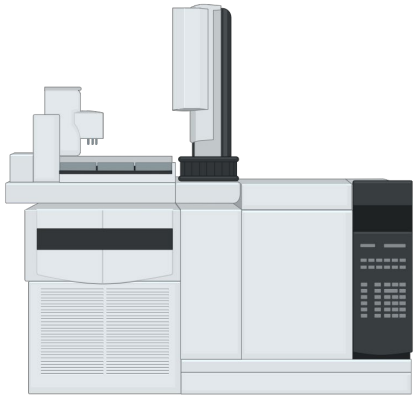
A new method to differentiate the origin of exhaled VOC







SESI-HRMS



GC

Data Analysis

1 Origin Stats

y_{1000h} = Period + Origin + Time + Origin*Time + (1 |Trt) + (1 |Cow)

2 Treatment Stats

y_{Origin} = Period + Time + Diet + His + Time*Diet + Time*His + Diet*His + Time*Diet*His + (1 |Cow)

3 Data visualization

Eq.1. $Lsm.Br./Lsm.Ex.$

Eq.2 $Sig = -\log_{10}(p.value)$

Eq.3 $FC = \log_2(Eq.1)$

4 m/z annotation

Previous studies.
HMDB
MetaboAnalyst®

Can we use methane to differentiate the origin of the VOC?

Origin *P* < 0.01
Time *P* < 0.01
Trt. *P* < 0.1
Time*Origin *P* < 0.01

Diff: -76%

Origin *P* < 0.01
Time *P* < 0.01
Trt. *P* < 0.05
Time*Origin *P* = 0.70

Diff: -24%

Metabolic energy lost was mainly accounted from breath CO_2

-80% **-76%** **-74%** **-69%** **-76%**

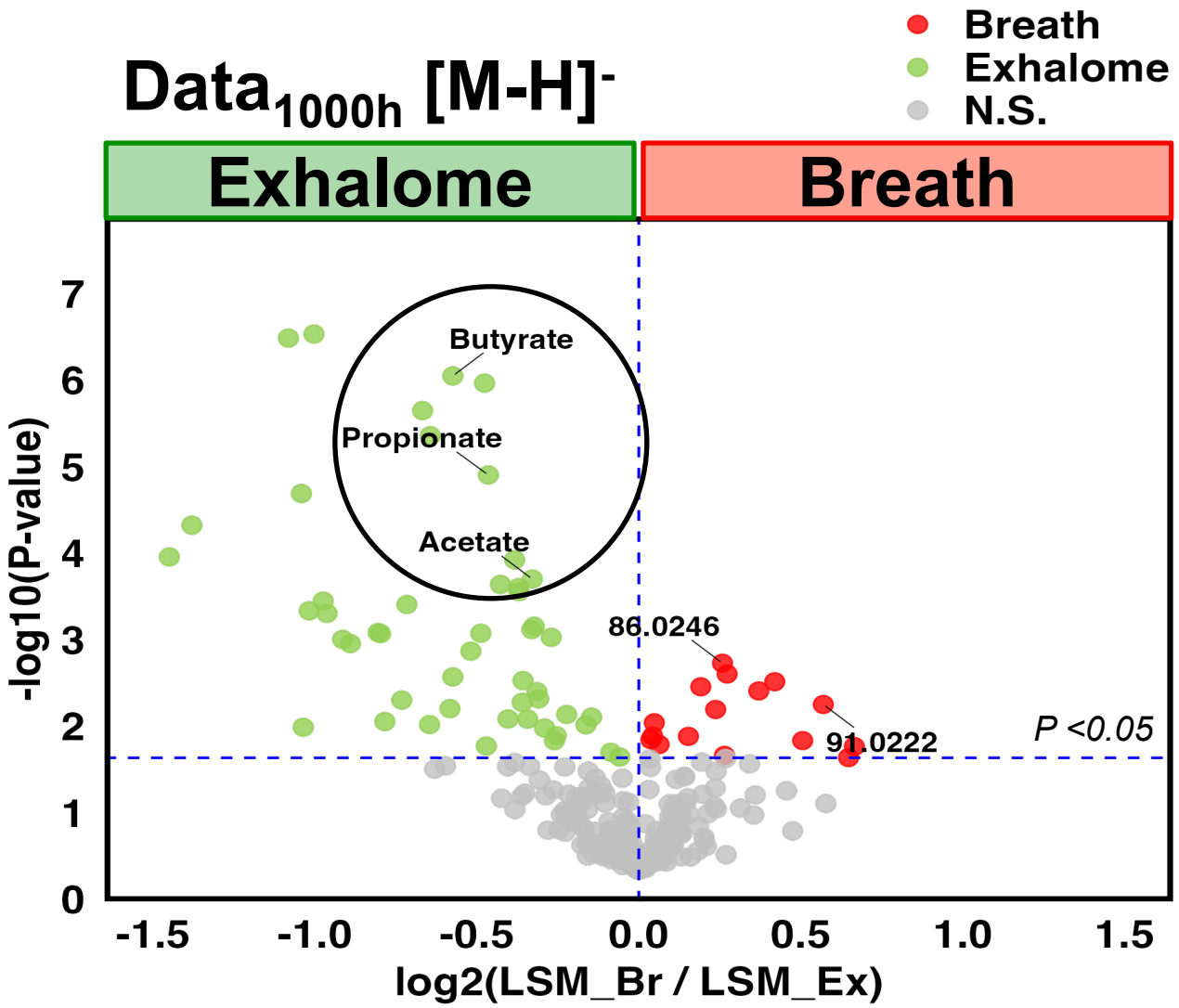
Higher VFA concentration was found in the exhalome

VFAs Difference

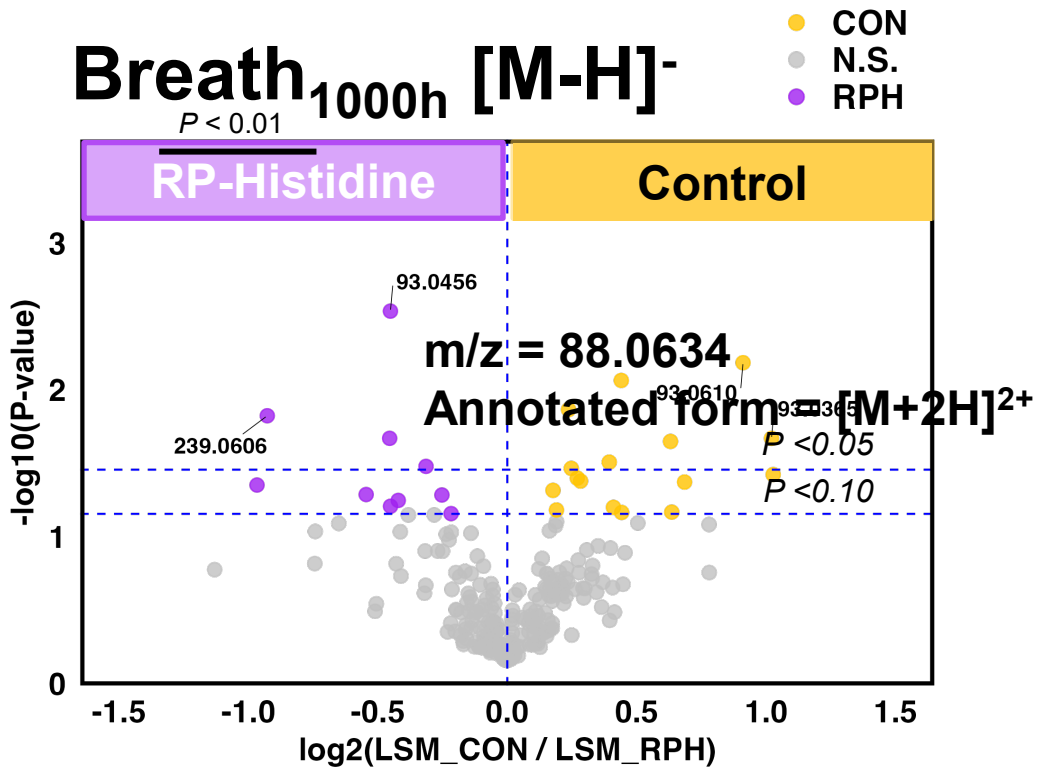
Acetate = 20.4%

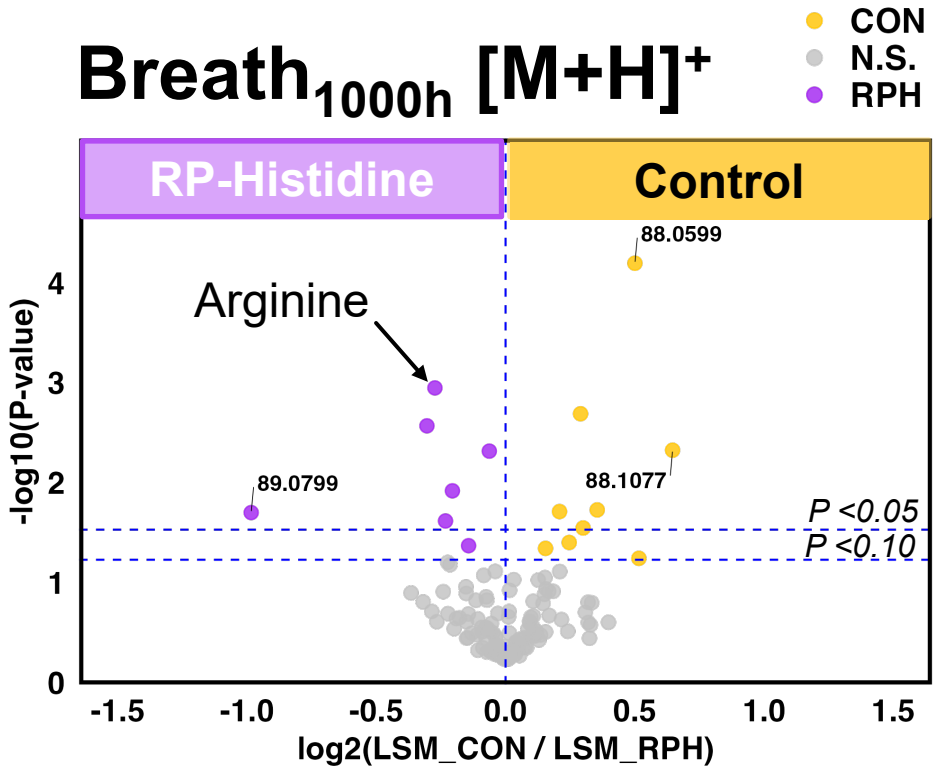
Butyrate = 32.8%

Propionate = 27.5%

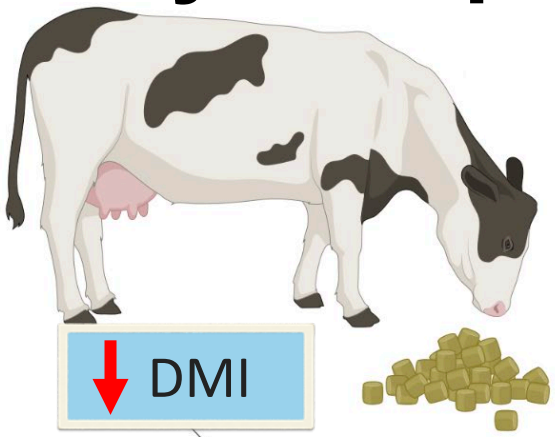


Breath profile changed by His supply





Dairy cow physiology

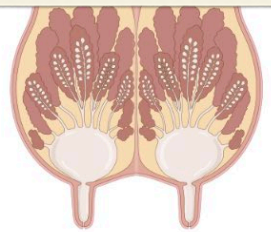


DMI

NEB

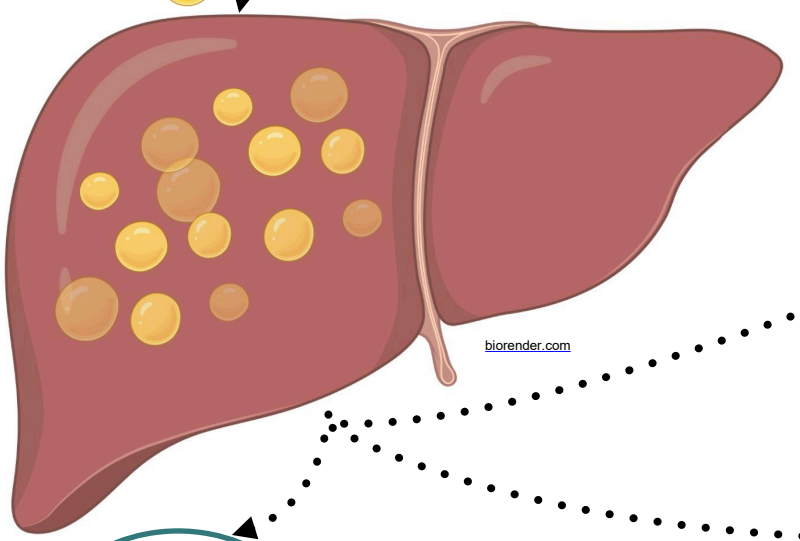
Glucose

Milk Production

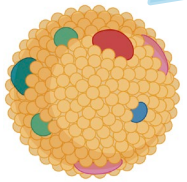


Lipolysis

FA Influx

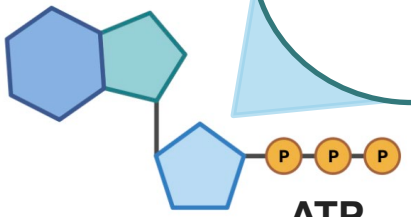


FA-
Re-ester.

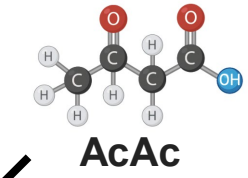


Ketones

Oxidation



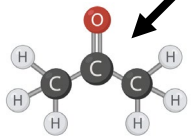
ATP



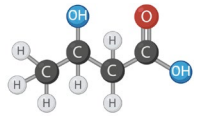
AcAc

NAD+

NADH



Acetone

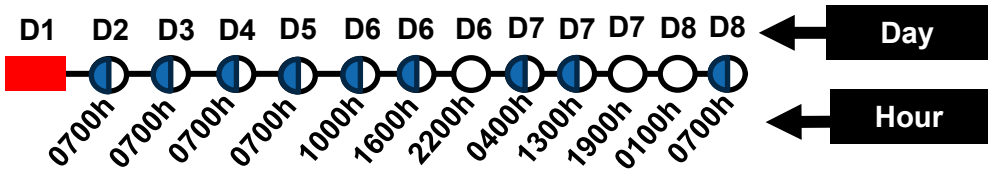


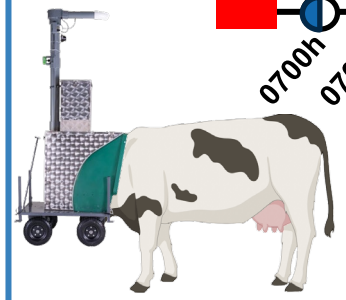
BHB

Bergman, 1971; White, 2015

Can we use breathomics to profile NEB?

2 *x* 2 Latin Square Design





*n = 12
*DIM = 100 ± 19.95
*MY = 43.4 ± 6.77

Gas chromatography (GC) and Secondary Electro Spray Ionization High Resolution Mass Spectrometer (SESI) analysis.

SESI analysis.

Start of caloric feed restriction.

Caloric feed restriction NEB model

60% hay forage diet.

Treatments:

1. Control
2. Negative Energy Balance (NEB)

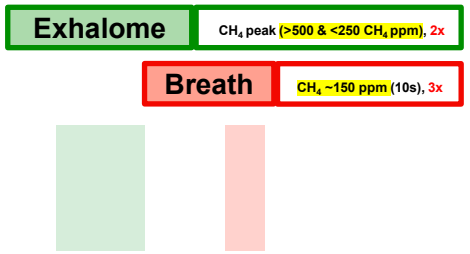
Table 1. Diet composition.

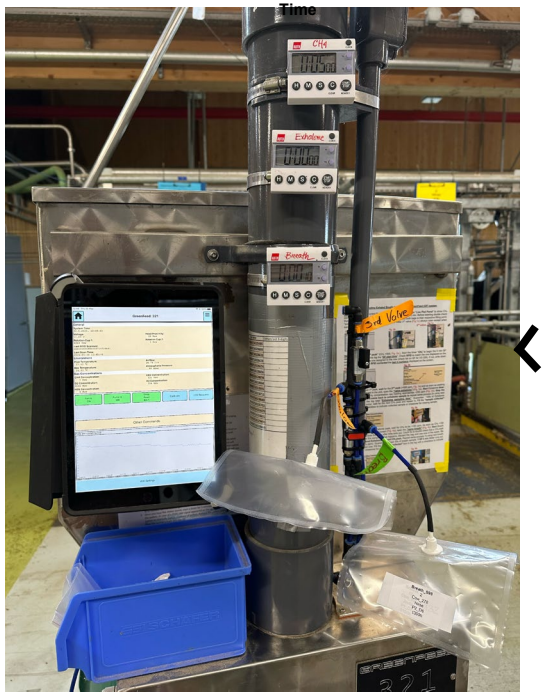
Feed Component	Control (DM%)	NEB (DM%)
Grass Hay	---	31
Wheat Straw	2	20
Alfalfa hay	12	9
Corn Silage	42	19
Grass Silage	16	7
Beet pulp	10	4
Concentrate Mix	10	10
Concentrate Mix 1	4	---
Concentrate Mix 2	4	---

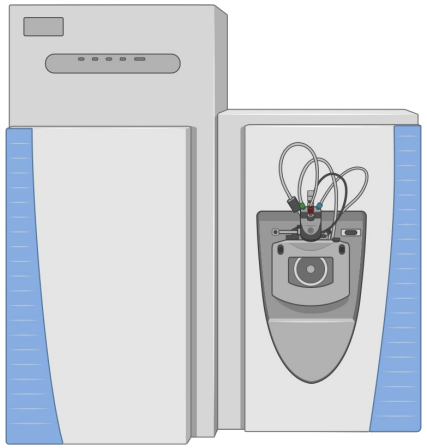
Table 2. Diet summary.

Diet	NDF (%DM)	Starch (%DM)	ME (Mcal/kg)	MP (%DM)
Control	37.3	16.8	2.64	8.71
NEB	51.1	7.9	2.49	7.21

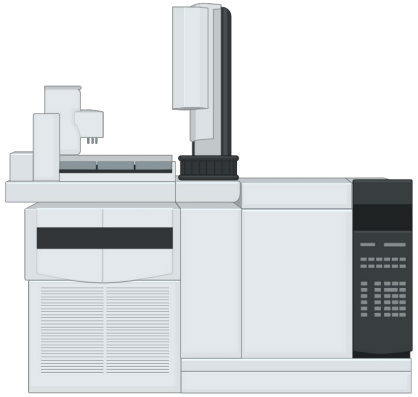
Can we use breathomics to profile NEB?







SESI-HRMS



GC

Data Analysis

1 Origin Stats

y_{1000h} = Period + Origin + Time + Origin*Time + (1 |Trt) + (1 |Cow)

2 Treatment Stats

y_{Origin} = Period + Time + Diet + Time*Diet + Time*Diet+ (1 |Cow)

3 Data visualization

Eq.1. $Lsm.Br./Lsm.Ex.$

Eq.2 $Sig = -\log_{10}(p.value)$

Eq.3 $FC = \log_2(Eq.1)$

4 m/z annotation

Previous studies.
HMDB
MetaboAnalyst®

Caloric restriction model induced NEB

Trt *P* < 0.01
Day *P* = 0.06
Day*Trt *P* < 0.01

Trt *P* < 0.01
Day *P* = 0.10
Day*Trt *P* < 0.05

SCK

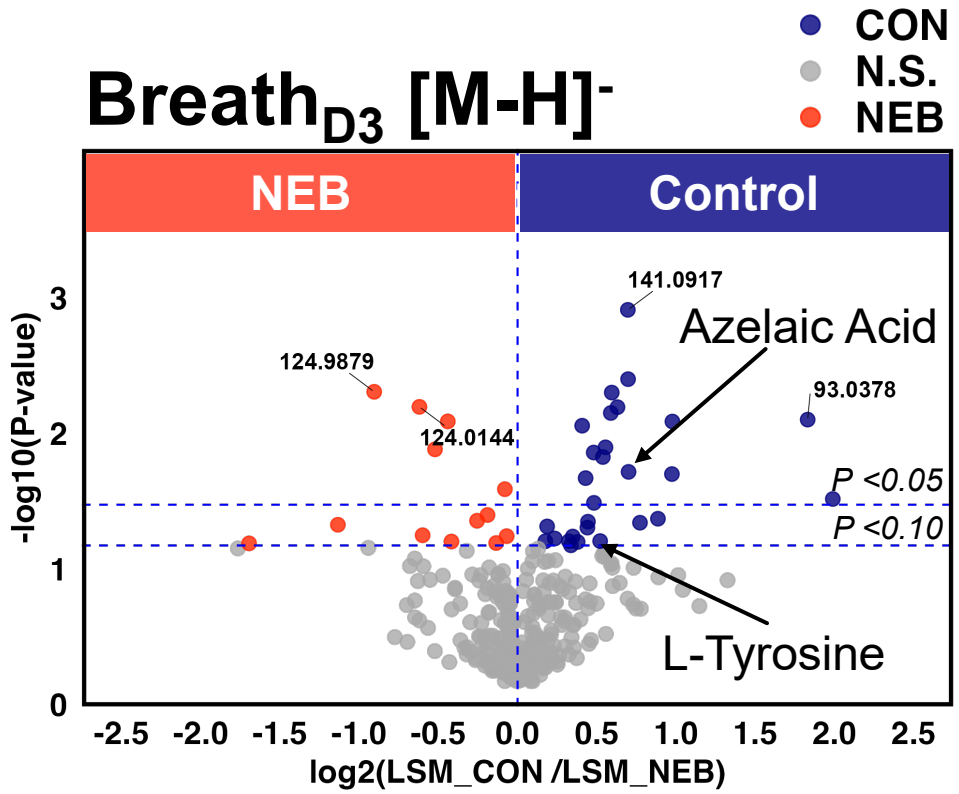
NEB = 1.06 mmol

Control = 0.94 mmol

Trt *P* < 0.01
Day *P* < 0.01
Day*Trt *P* = 0.12

Trt *P* < 0.05
Day *P* < 0.05
Day*Trt *P* = 0.35

Breath profile revealed changes in the energy metabolism



$P < 0.05$

$P < 0.10$

Azelaic Acid
m/z = 187.0978
Annotated form = [M-H]$^-$

L-Tyrosine
m/z = 180.0666
Annotated form = [M-H]$^-$

Taking home messages

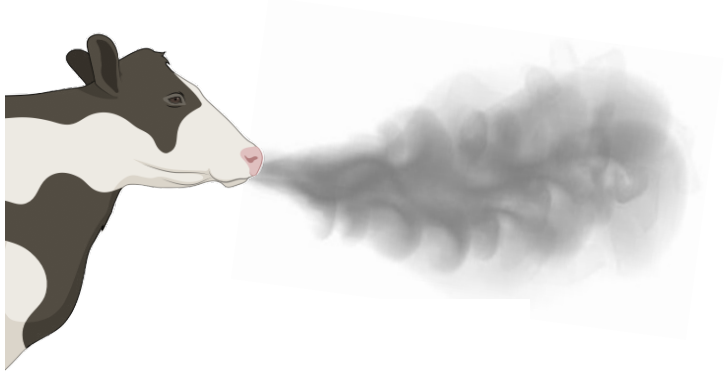
- Methane biomarker allowed us differentiate up to 80% of the blending effect of eructations on breath.
- Our method to differentiate the VOCs' origin enabled the identification of metabolic changes in dairy cows under different nutritional strategies.
- The absence libraries for annotating compouds identified thorugh SESI-HRMS analysis limited the ability to fully understand the metabolic changes associated to different nutrional strategies in dairy cows.

AgroVet Strickhof

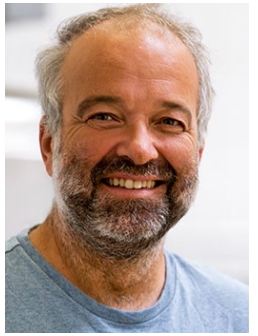
D CHAB

Agroscope

Zurich Exhalomics



Thank You!



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