VolAER

Volatolomique de l'air exhalé par le ruminant

The volatolomic signature differs between primiparous and multiparous cows across different matrices

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INP TOULOUSE PURPAN ÉCOLE D'INGÉNIEURS

LESAFFRE Phileo by Lesaffre

Introduction

Nutritional and physiological research → non-invasive biomarkers of the metabolic status

Metabolomics → potential development of biomarkers of different phenotypes

- ✓ Feed efficiency
- ✓ Ketosis, acidosis
- ✓ Product quality

(Saleem et al., 2012; Foroutan et al.,2020; Goldansaz et al. , 2017)

Volatolomics arises as an alternative within metabolomics approchaes

The profile of volatile organic compounds (VOC)

GC-MS targeted analysis → succesfull discrimination of contrasting diets (Silberberg et al., 2018; Eichinger et al., 2023).

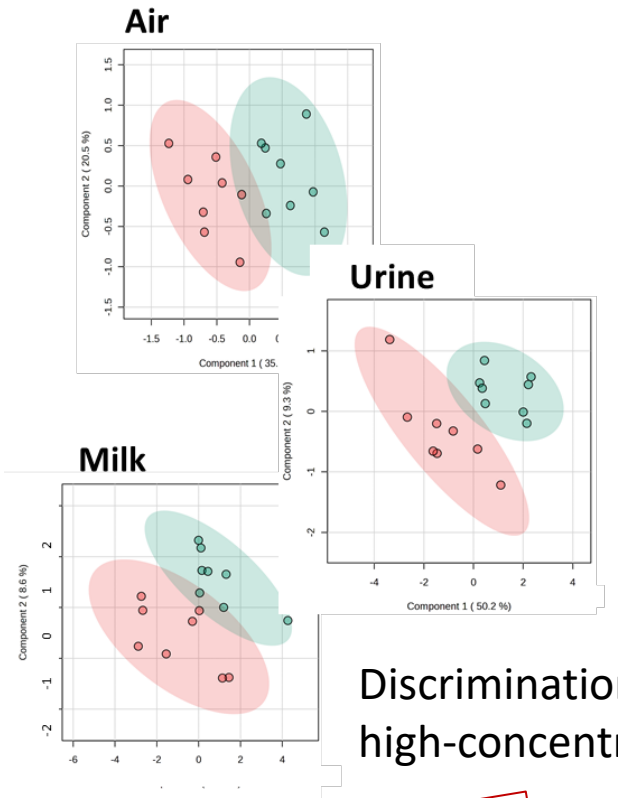
- ✓ Time consuming
- ✓ No feasible at field conditions

Single Ion Flow Tube - Mass Spectrometry (SIFT-MS)

- ✓ No sample preparation
- ✓ Easy and cheap

- ✓ Biomarkers of human and pathologies (Dharmawardana et al. 2020; Segers, et al., 2021)
- ✓ Succesfully used with different matrices, e.g.: urine, sweat or breath (Smith et al. 1999; Catala, et al., 2020; Belluomo, et al., 2021)

Single Ion Flow Tube - Mass Spectrometry (SIFT-MS)



Discrimination of high-fibre vs. high-concentrate diet

Preliminary results

Session 89
Wednesday 4th

Other sources of effect on the volatolomic profile of dairy cows?

Primiparous vs. multiparous cows

- ✓ physiological maturity
- ✓ milk yield
- ✓ behaviour
- ✓ metabolic differences (Wathes et al., 2017; Adrien et al., 2012; Saqib et al., 2018)

Should we take into account the parity when performing non-targeted volatolomics?

Is it possible to discriminate primiparous vs. multiparous (2nd, 3rd lacatation) cows?

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Hypothesis

The metabolic differences between primiparous and multiparous cows affect the profile of volatile organic compounds (VOC) which can be determined by untargeted volatolomics based on SIFT-MS

Objective

To assess the volatolomic differences between primiparous and multiparous cows based on SIFT-MS untargeted volatolome of breath, ruminal liquid, feces, urine, milk and sweat.

Materials and Methods

PRIM

n = 10

1st lactation

73 ± 11 DIM

22.3 ± 3.31 kg/d milk

618 ± 41 kg BW

1.5 ± 0.20 BCS

MULT

n = 6

2nd lactation

76 ± 11 DIM

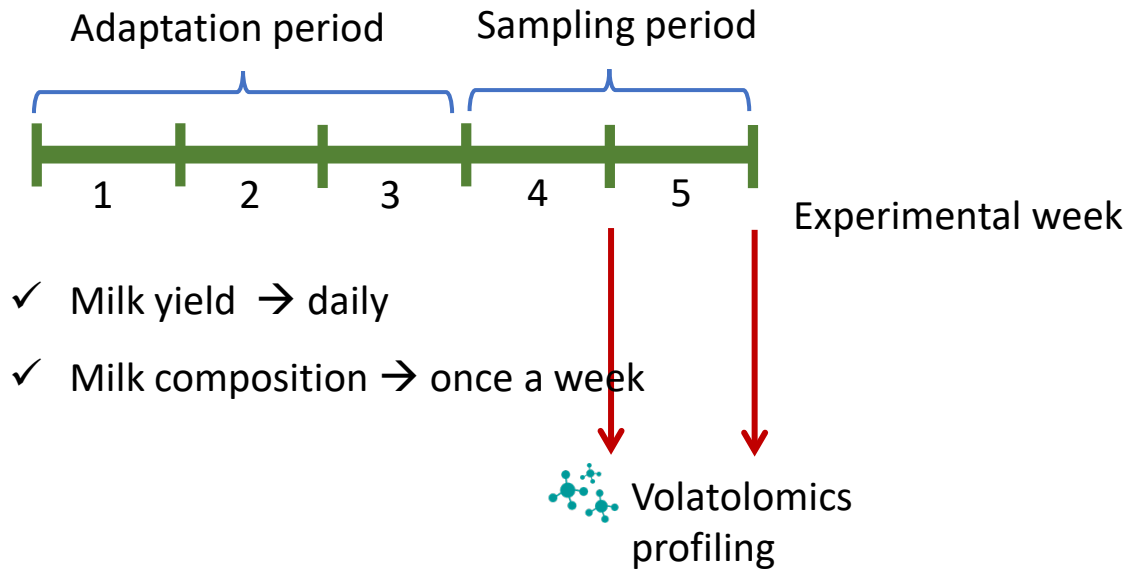
23.0 ± 1.59 kg/d milk

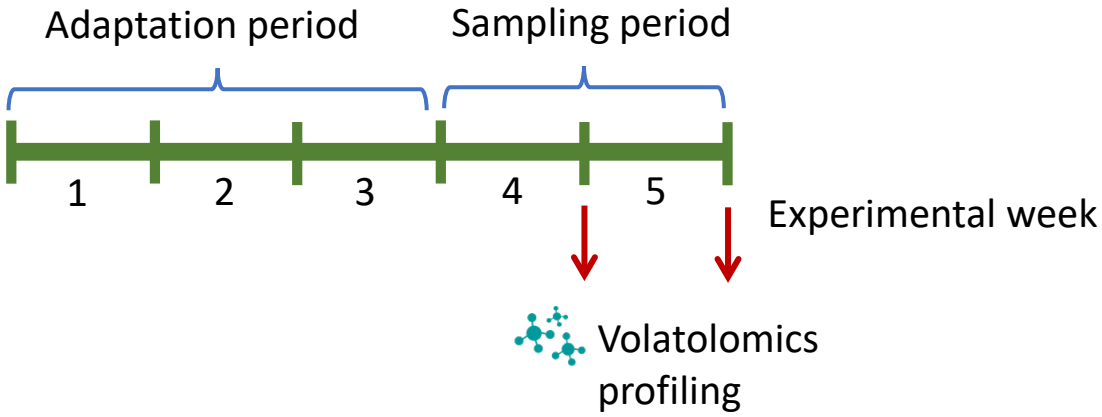
642 ± 49 kg BW

1.4 ± 0.20 BCS

Forage: 76 % DM

Concentrate: 24 % DM

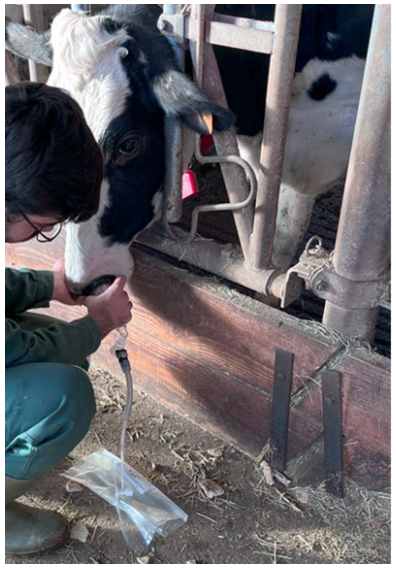


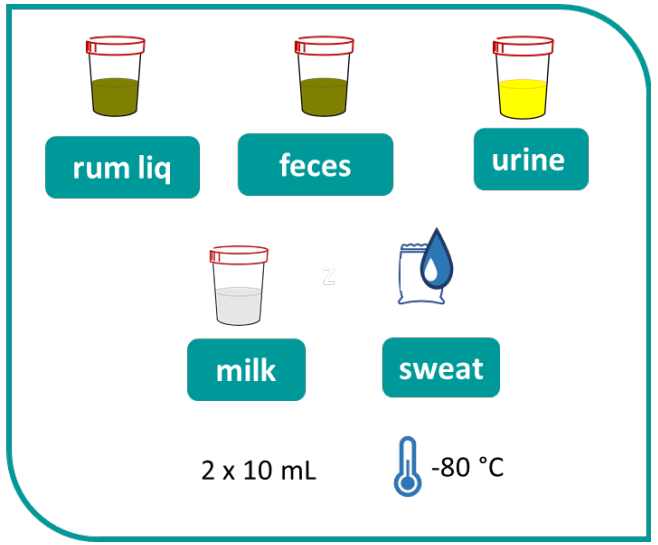


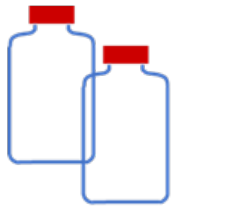
breath

T° amb, 24h, SIFT-MS

TLS







breath

T° Amb (25 °)

rum liq

feces

urine

milk

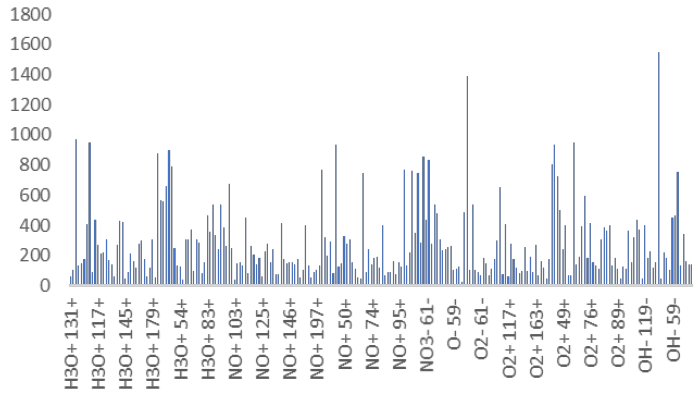
sweat

2 x 10 mL

-80 °C

Heat (37 °, 30 min)
Headspace

✓ 1860 ions measured in 10 minutes



Statistical analyses

1. Productive data

DMI and performance as repeated measures (mixed model)

- Parity, week (4, 5), Parity × week → fixed effects
- Cow(parity) → random effect
- AR(1) as the covariance structure

2. Volatolomics data

1860 ions

Each week and matrix → analysed separately

$Q^2 \geq 0.5$ → valide, predictive
$0 < Q^2 < 0.5$ → valide non predictive
$Q^2 < 0$ → non valide

Results and discussion

Table 1. DMI and productive performance in primiparous and multiparous cows in weeks 4 and 5

Trait	LS-means		SEM	P-value		
	PRIM	MULT		Diet	Week	Diet × Week
DMI (kg DM/d)	20.6	21.6	0.64	0.288	0.017	0.475
Milk yield (kg/d)	21.7	21.3	0.95	0.787	0.301	0.194
Milk fat (g/100 g)	3.37	3.84	0.144	0.042	0.004	0.727
Milk protein (g/100 g)	2.90	2.87	0.520	0.668	< 0.001	0.832

PRIM MULT

No major differences in terms of production and DMI

1180 ions effectively quantified:

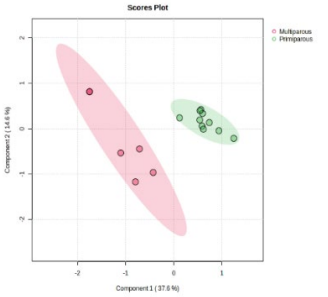
193 – 451 → according to the matrix

PRIM

MULT

Week 4

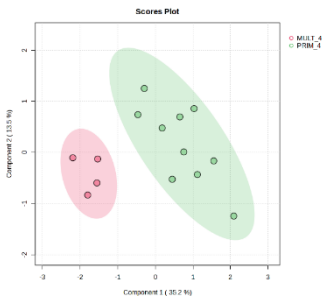
Air



$R^2 = 0.96$

$Q^2 = 0.83$

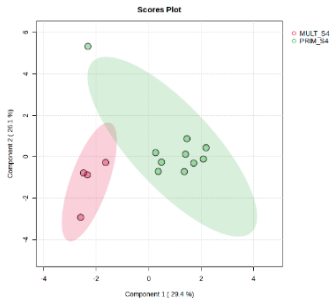
Ruminal liquid



$R^2 = 0.71$

$Q^2 = 0.59$

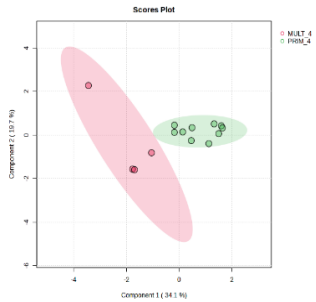
Feces



$R^2 = 0.64$

$Q^2 = 0.14$

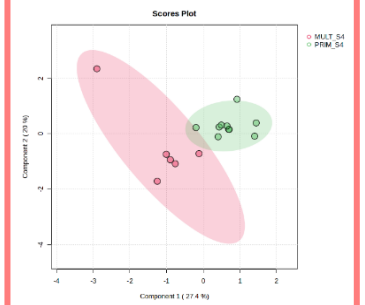
Urine



$R^2 = 0.74$

$Q^2 = 0.44$

Sweat

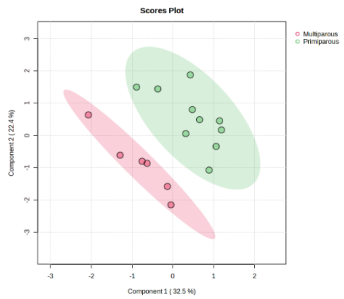


$R^2 = 0.66$

$Q^2 = 0.37$

Week 5

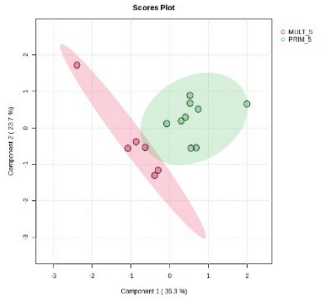
Air



$R^2 = 0.48$

$Q^2 = -0.11$

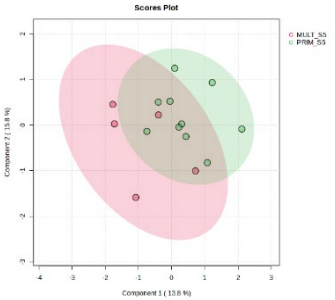
Ruminal liquid



$R^2 = 0.76$

$Q^2 = 0.37$

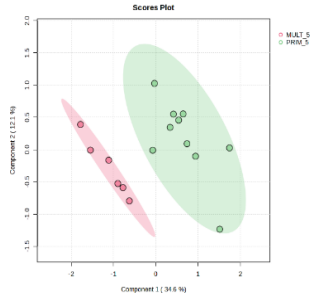
Feces



$R^2 = 0.34$

$Q^2 = -0.93$

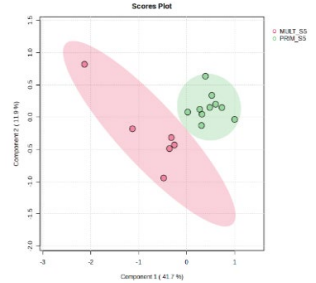
Urine



$R^2 = 0.74$

$Q^2 = 0.58$

Sweat



$R^2 = 0.86$

$Q^2 = 0.59$

Take-home messages

- ✓ The **parity impact** the **volatolomics** profile of different matrices
- ✓ Matrices related with the **host metabolism** → better potential to **discriminate the parity class**
- ✓ The **breath** is possibly the most sensitive matrix to environmental interferences
  - → Integrate current developments to **refine the breath sampling** ? (Islam et al., 2023)
- ✓ Ruminal liquid, urine and sweat → better consistency between sampling periods
- ✓ The results based on multiparous or primiparous only cannot be extrapolated between parities
  - → attention to the robustness of conclusions for field application

Thank you

REAGENT ION SELECTION

Microwave plasma

R^+ R^-

O_2^+ NO_3^-

H_3O^+ NO_2^-

NO^+ O^-

O_2^-

OH^-

Quadrupole mass filter

Select one R^- or R^+

ANALYTE SOFT IONISATION

Sample inlet

Flow tube

Carrier gas inlet

$$R^{(+\text{ or }-)} + A \xrightarrow{k} P^{(+\text{ or }-)} + N$$

Reagent ion — Analyte — Product ion — Neutral product

IONS DETECTION

Quadrupole mass filter

Particle multiplier

Scan mode or SIM mode

$$[A] = \gamma \frac{[P^{(+\text{ or }-)}]}{[R^{(+\text{ or }-)}]k}$$

γ = instrument calibration factor