

> Dairy cows' diet discrimination through SIFT-MS-based volatolome of different matrices

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Introduction

Nutritional research and precision nutrition for ruminants require following up the rumen functioning

Up to now → invasive techniques (ruminal canula, oesophageal probe, rumenocentesis)

Non-invasive biomarkers of rumen functioning and host response are needed

The research problem

Metabolomics → potential development of biomarkers

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

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

Sub-acute ruminal acidosis (**SARA**)

→ high prevalence, silent, welfare and production constrain

→ alterations of rumen microbiota and fermentation

→ altered volatile fatty acid (**VFA**) and volatile organic compounds (**VOC**)



Volatolomic profile of
ruminal liquid, eructated air

→ Inflammation and oxidative stress of the host ruminant

→ altered VOC



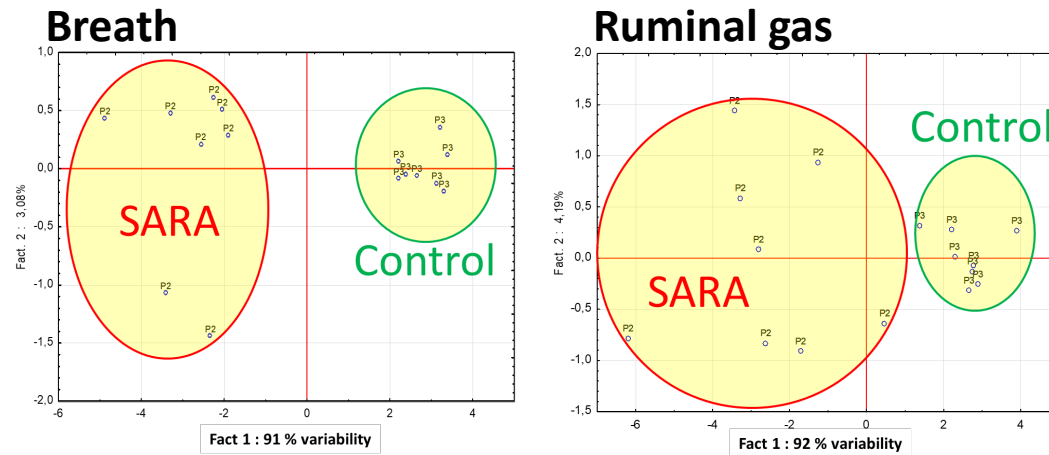
Volatolomics profile of the headspace of
breath, feces, urine, milk, sweat

Good model for developing rumen functioning biomarkers and host metabolic responses

Silberberg et al. (2018)

→ determination of some VOC through GC-MS

→ 4 lactating cows



- ✓ Time consuming
- ✓ No feasible at field conditions

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

- ✓ No sample preparation
- ✓ Easy and cheap
- ✓ 1860 ions measured in 10 minutes



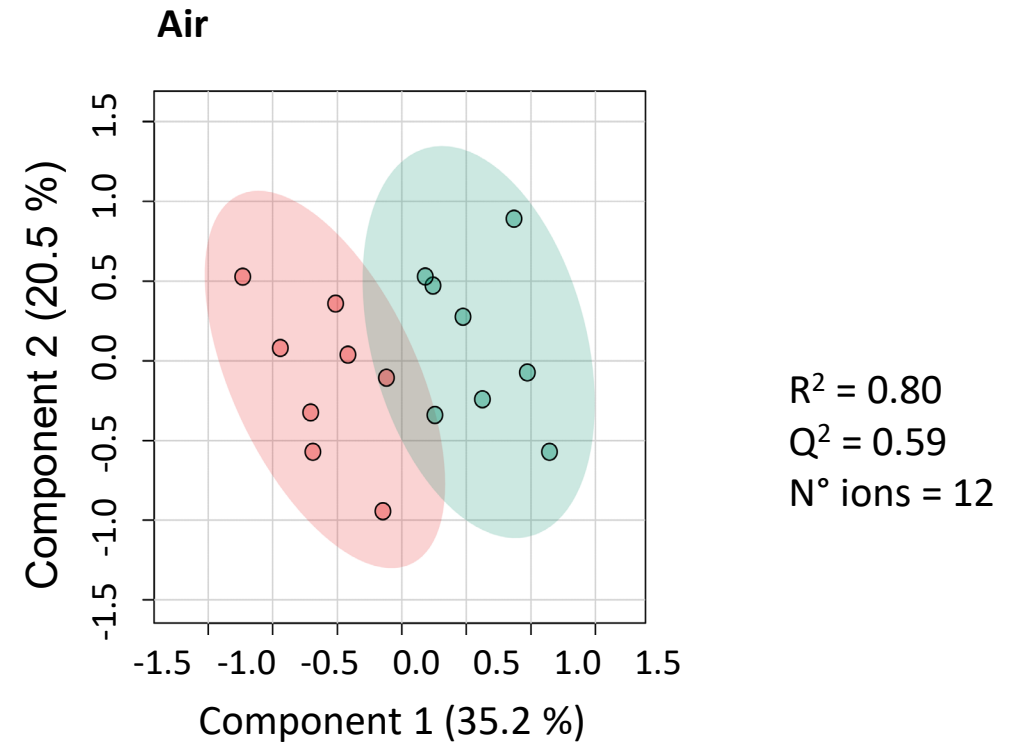
Biomarkers of pathologies:

- Cancer (Dharmawardana et al. 2020)
- Asthma (Segers, et al., 2021)
- Epilepsy (Catala, et al., 2020)

Successfully used with different matrices

- Urine (Smith et al. 1999)
- Feces (Smith et al. 2000)
- Sweat (Catala, et al., 2020)
- Breath (Belluomo, et al., 2021)

A model of acidogenic diet: high-fibre vs. high-concentrate



Breath volatolomics profile discriminated the diet

Jorge-Smeding et al. (2023)



Hypothesis:

- The alterations of **rumen functioning** and **host metabolism** triggered by a high-starch diet causing a state of **SARA** alter the **volatolomic** profile of the **rumen liquid, urine, feces, milk** and **sweat**.
- This alterations of the **volatolomic** profile of different matrices' head space can be determined through **SIFT-MS** and serve a source for ruminal functioning/diet **biomarkers** development.

Objective:

- To test the ability of **SIFT-MS**-based volatolomics profiles of ruminal liquid, feces, urine, milk and sweat to yield **predictive models** of two **diets** based on the same feedstuffs but **contrasting acidogenic potential** (high fibre vs. high starch).

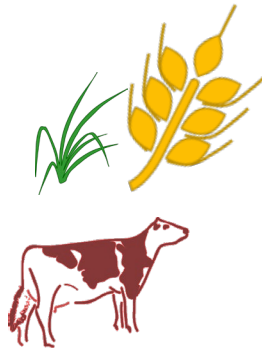
Materials and Methods

Same feedstuffs but combined in different proportions



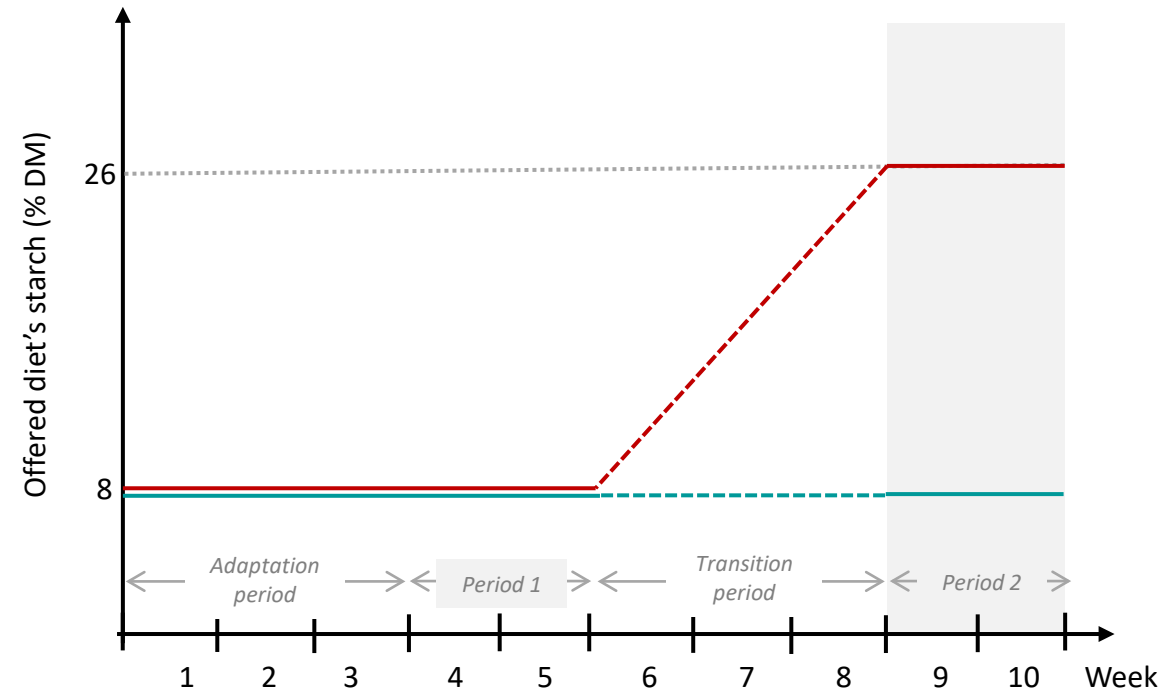
HFIBRE

n = 8



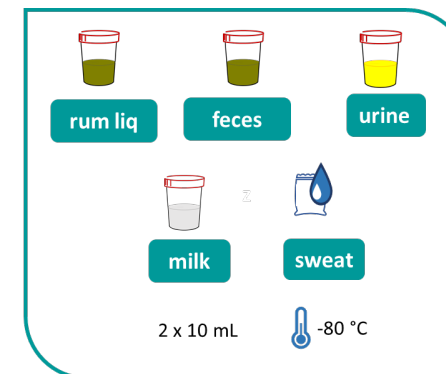
HSTARCH

n = 8



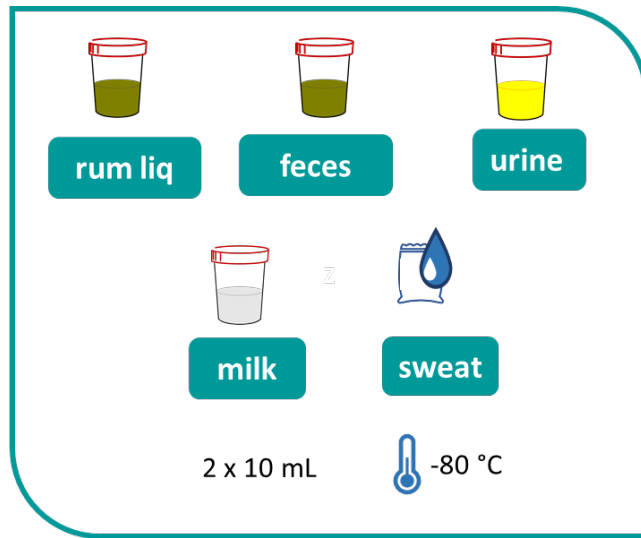
Validated diet challenge → expected responses achieved

- ✓ pH altered
- ✓ Alterations in protozoa count and VFA concentrations



Volatolomics
profiling

SIFT-MS analysis



Defrosted in a glass cotevant (37 °C, 10 mins)



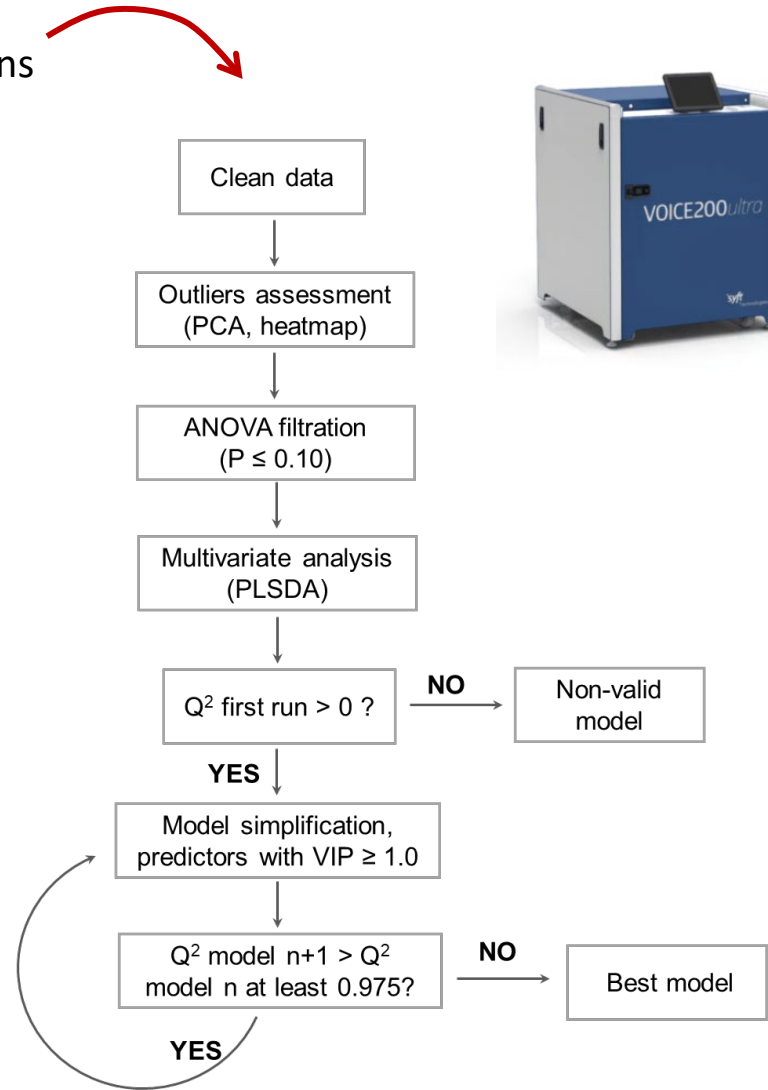
Heated (45 °C, 30 mins)



Headspace analysis

Statistical analysis

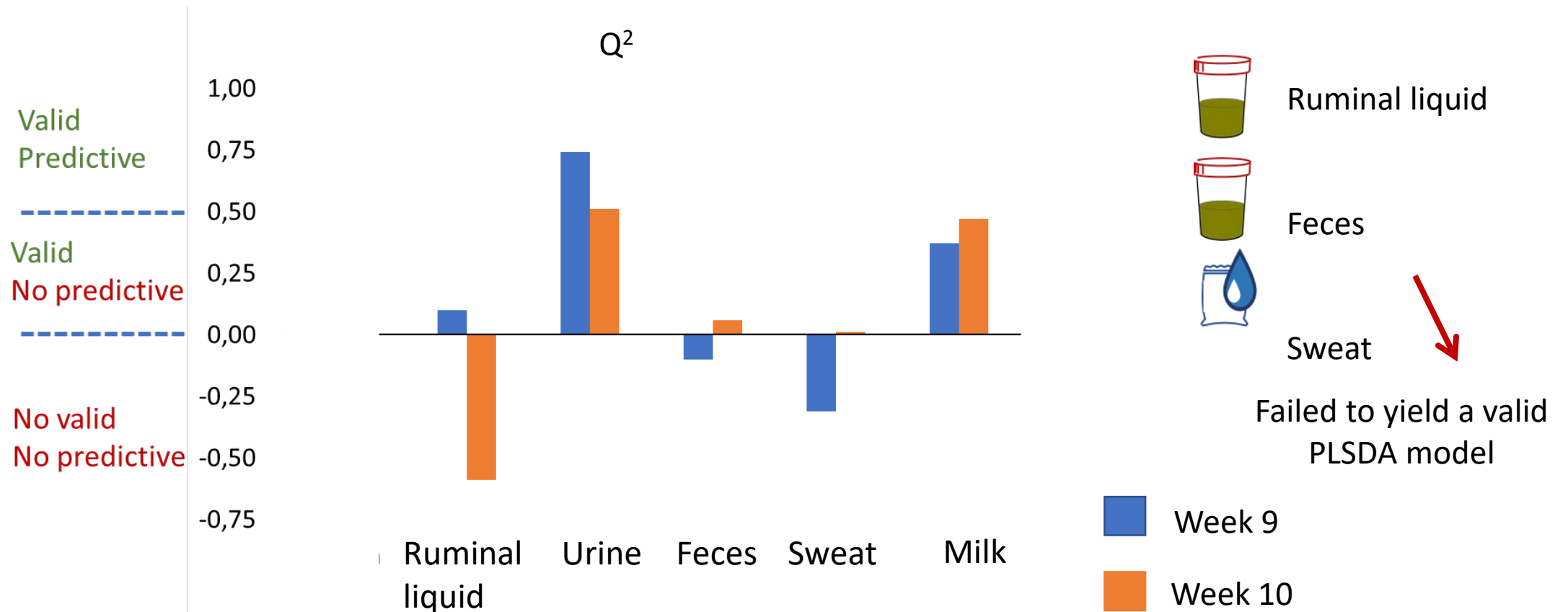
1860 ions



Results and discussion

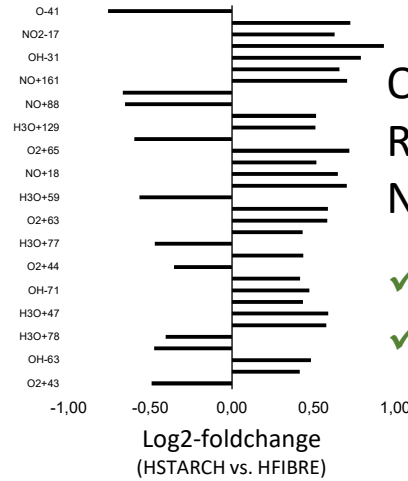
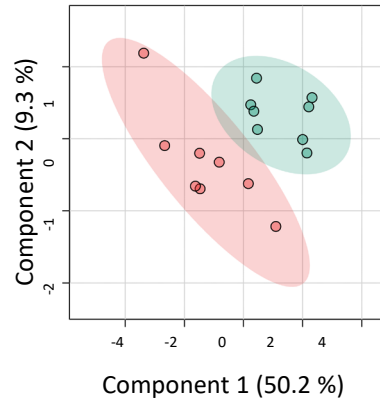
1180 ions effectively quantified

Final datasets: 193 – 451 → according to the matrix



Week 9

Urine

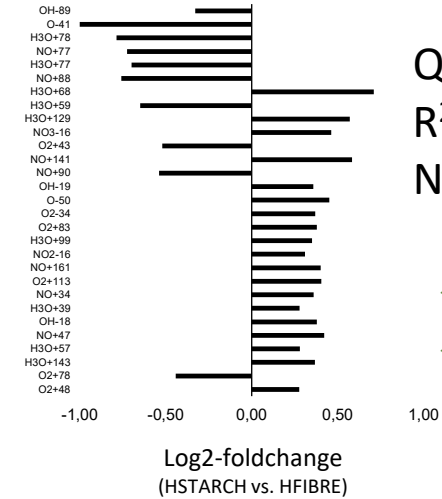
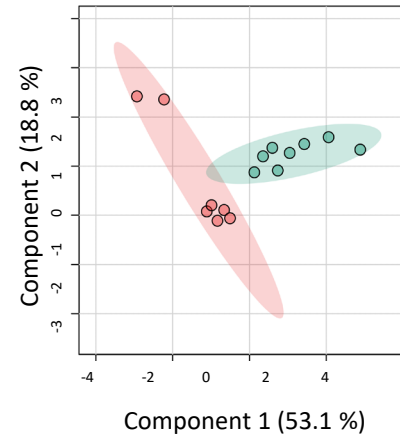


$Q^2 = 0.74$
 $R^2 = 0.91$
 $N^\circ \text{ pred} = 33$

✓ Valide
 ✓ Predictive

Week 10

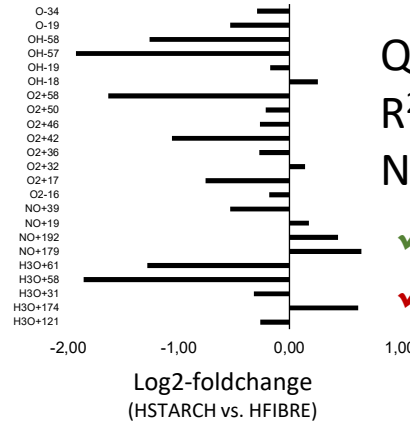
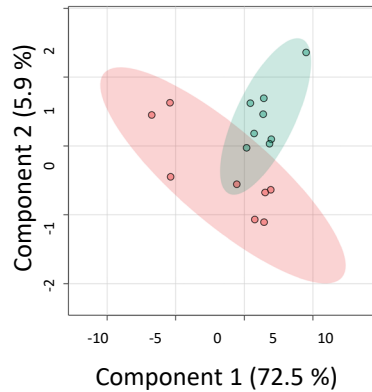
Urine



$Q^2 = 0.51$
 $R^2 = 0.66$
 $N^\circ \text{ pred} = 29$

✓ Valide
 ✓ Predictive

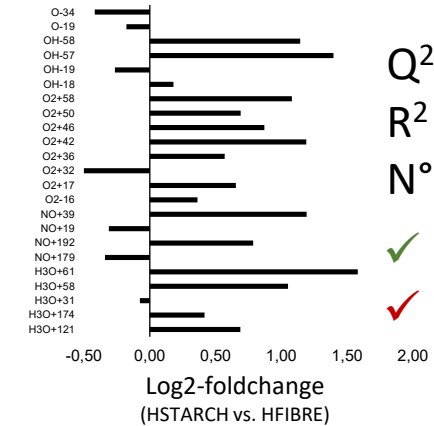
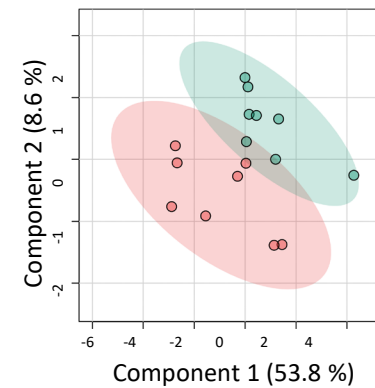
Milk



$Q^2 = 0.37$
 $R^2 = 0.67$
 $N^\circ \text{ pred} = 23$

✓ Valide
 ✓ No Predictive

Milk



$Q^2 = 0.47$
 $R^2 = 0.82$
 $N^\circ \text{ pred} = 23$

✓ Valide
 ✓ No Predictive

Interesting matrices to consider in future studies:

Milk

- lower predictive ability
- improved Q^2 in week 10 → differences in milk composition (protein, fat:protein content)

Urine

- less interferences
- reflecting the host metabolism
- VOC reflecting oxidative stress and inflammation caused by SARA?
- lower impact of digestive process than in others matrices

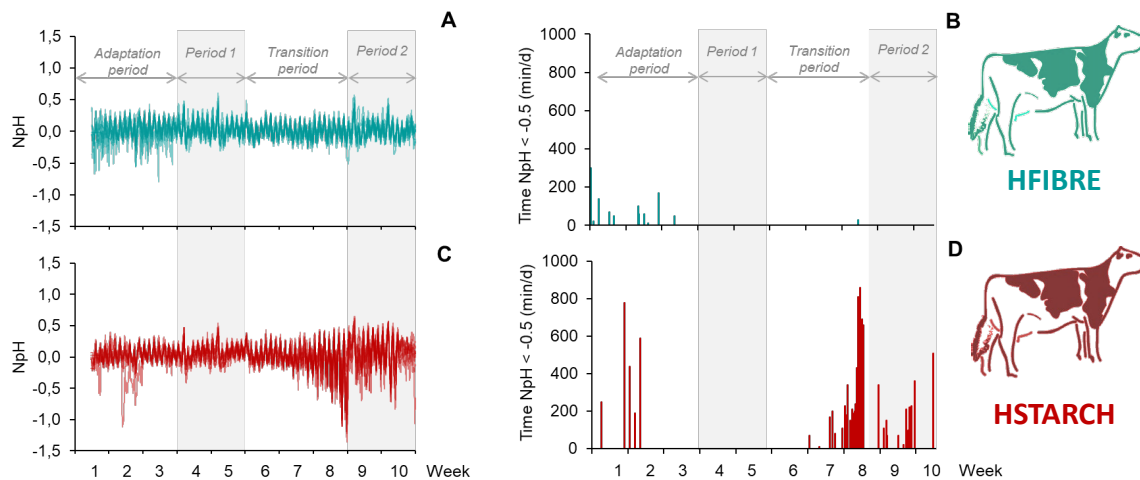
Parity effect → also urine
the most reliable matrix

Session 13:
Sensing physiology



Thank you





Normalized pH (NpH) and time with NpH < -0.5



Higher risk of SARA

Validated dietary challenge

→ expected responses

