

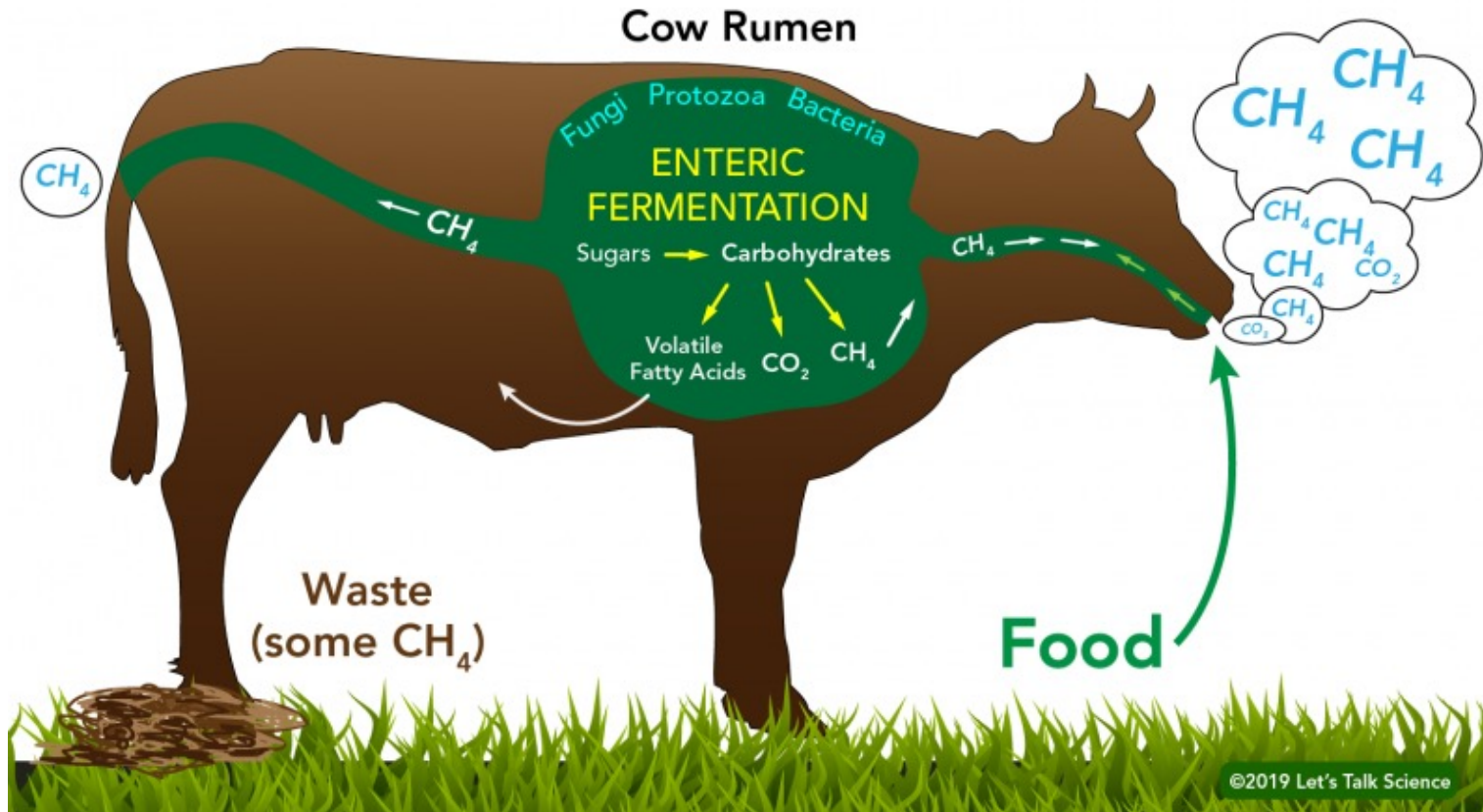


Exhalomics as a non-invasive method for assessing rumen fermentation in dairy cows

Zakirul Islam, Susanna Räisänen, Stamatios Giannoukos, Fabian Wahl, Renato Zenobi, Mutian Niu

Animal Nutrition Group, ETH Zurich

Unique Role of Ruminants in Agri-Food

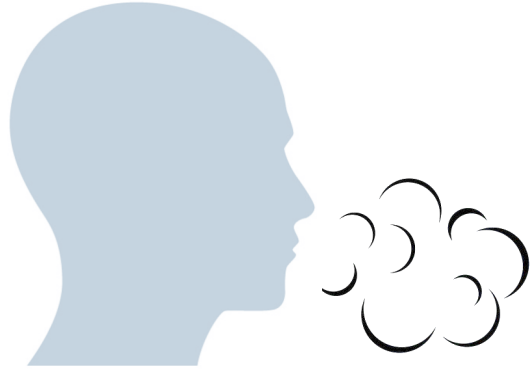


- Rumen fermentation and microbial activity are key

Other Non-invasive Alternative Approaches?



Exhalomics in Human and Ruminant Research



- Human breath biomarker-based diagnosis

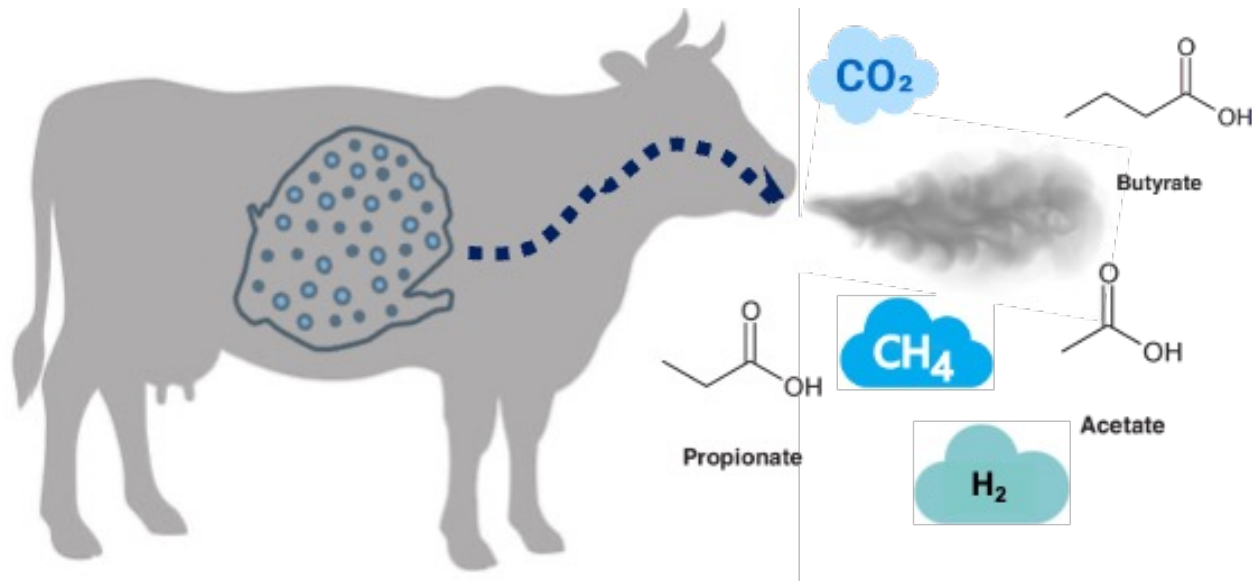


- Diagnostics of asthma in children

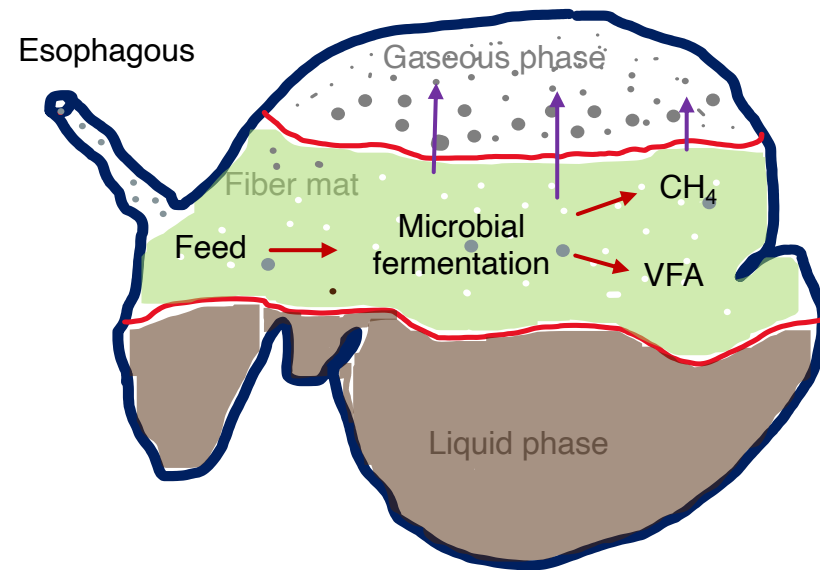
- Ketosis detection in exhalomes using GC-MS (Dobbelaar et al., 1996)
- Effect of eructation on some exhaled volatile compounds profiles (Oertel et al., 2018)
- Similar molar proportions of VFA in rumen gas and liquid samples (Dewhurst et al, 2001)

- **Research gap:** key rumen fermentation parameters in exhalome – VFAs remain largely unexplored

Bovine Exhalomics for Animal Research



Bovine “**breath**” or **Exhalome**:
lungs + rumen



- **Eructation**: regulate the release of gases from rumen into the atmosphere

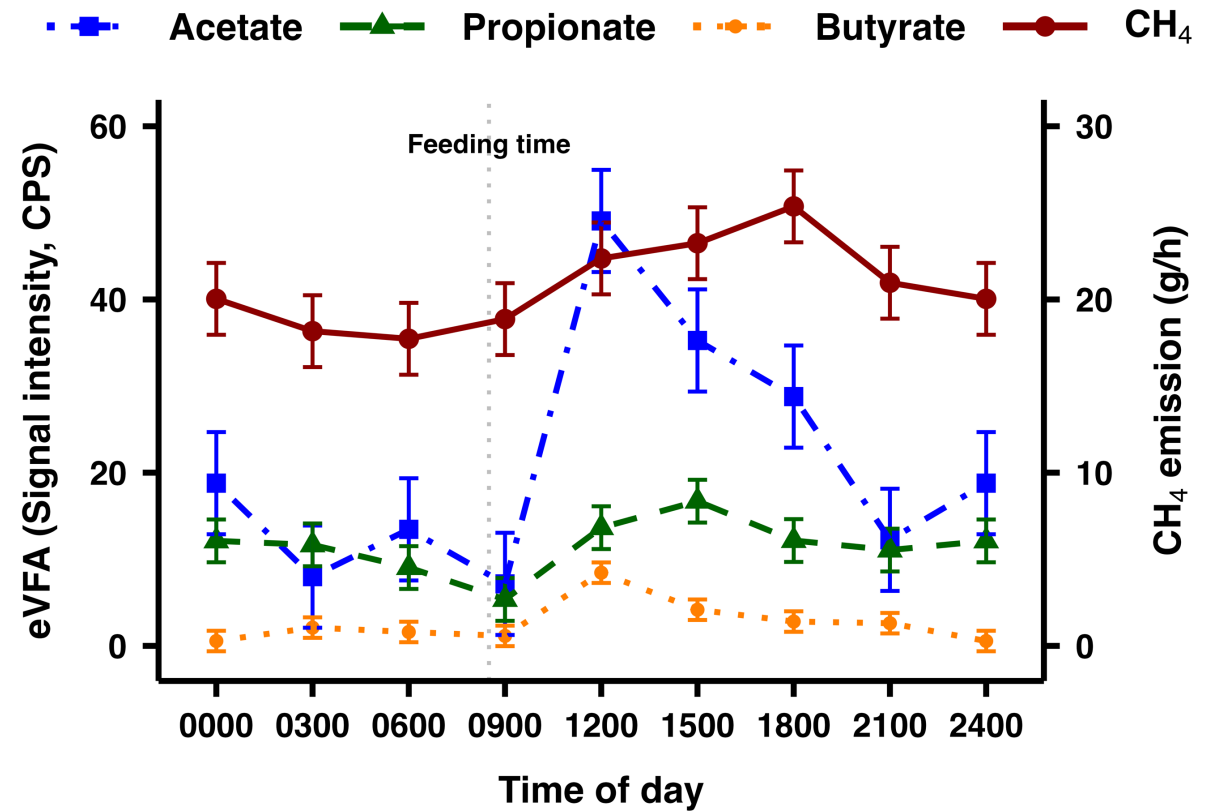
Daily Pattern of Exhaled Volatile Fatty Acids (eVFA)



GreenFeed (head chamber)



SESI-HRMS

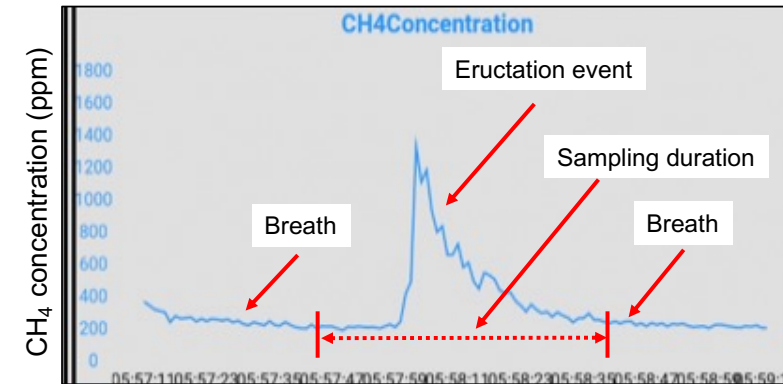


- Concentrations of exhaled acetate and butyrate increased rapidly right after feeding

**Can exhalomics replace
rumen sampling?**

Objective: To Validate the Exhaled VFA as a Proxy for Rumen VFA

- Rumen-cannulated cows
 - ✓ $n = 4$
- Design: 3-period Switchback (ABA/BAB)
- Diet treatments
 - ✓ High-starch (16% of DM)
 - ✓ Low-starch (6% of DM)
- Sample collection
 - ✓ Eight times over 2 days to represent every 3-h across the day



Exhalome sampling



SESI-HRMS for eVFA



Rumen fluid sampling



HPLC for rVFA

Statistical Analysis

- Prediction of VFA in gas phase using Henry's Law
- Mixed model with repeated measures using R

$$Y_{ijk} = \mu + CS_i + P_j + D_d + M_c + D_d \times M_c + T_k + CO_e + e_{ijk}$$

- Random effect of cow nested in sequence
- Random effect of time
- Fixed effect of period
- Fixed effect of diet
- Fixed effect of VFA measurement method
- Fixed effect of diet × method interactions
- Fixed carryover effect

$$Y_{ijk} = \mu + CS_i + P_j + M_d \times T_c + CO_e + e_{ijk}$$

- Method × time of day interactions

No Interactions Between VFA Measurement Method and Diet

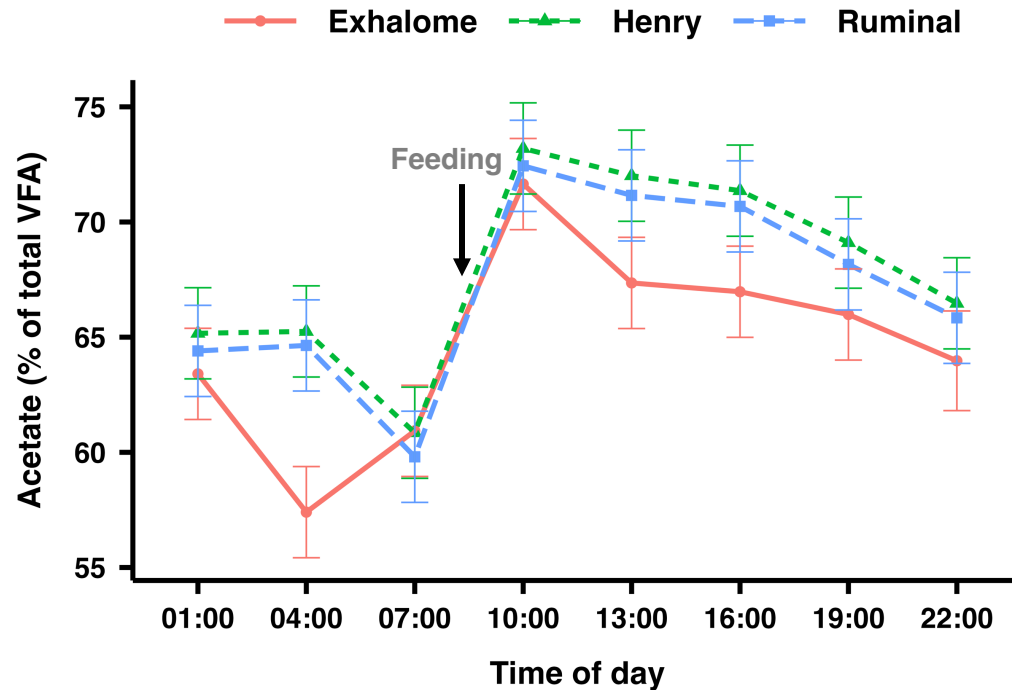
	Least Square Means						SE	P-value		
	HS-E	LS-E	HS-R	LS-R	HS-H	LS-H		Diet	Method	Diet × Method
% of total VFA										
Acetate	63.6	61.3	65.9	65.2	66.7	65.9	1.51	0.29	< 0.05	0.61
Propionate	27.8	28.9	23.0	24.5	23.7	25.3	1.17	0.21	< 0.05	0.94
Butyrate	7.93	8.72	10.3	10.4	8.97	8.84	0.551	0.62	< 0.05	0.36
A:P	2.36	2.25	3.02	2.94	2.95	2.87	0.195	0.61	< 0.05	0.98

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

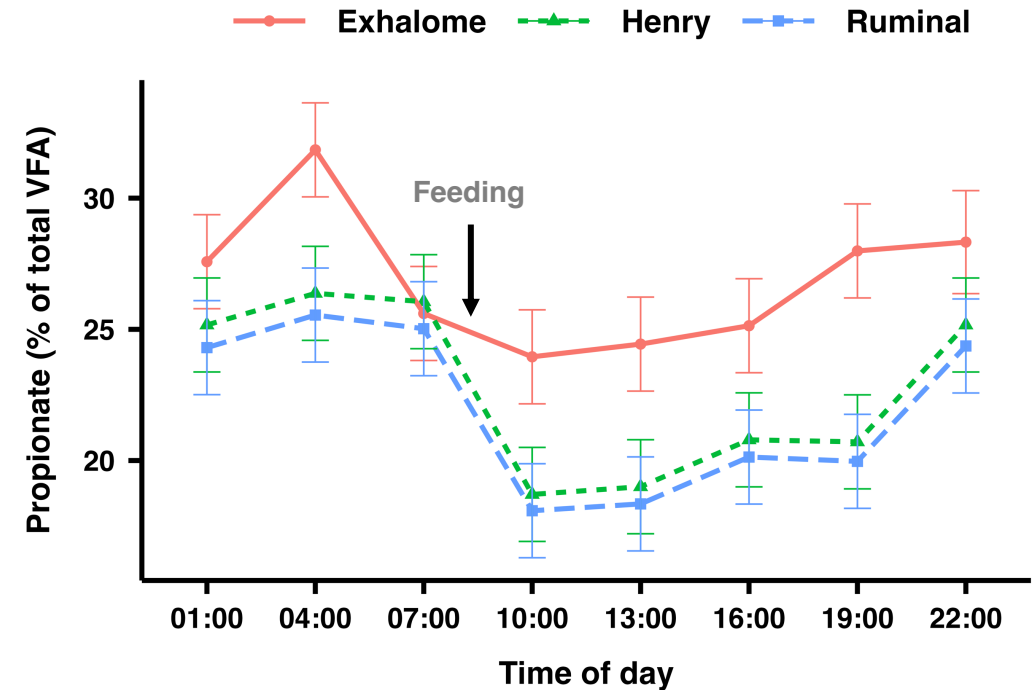
- No interactions for all rumen fermentation parameters
- Similar numerical changes for acetate, propionate, and A:P, might be because of lack of power

Ruminal vs. Exhaled VFA in 3-h Intervals - High-starch Diet

Time, $P < 0.05$
 Method, $P < 0.05$
 Time*Method, $P = 0.89$



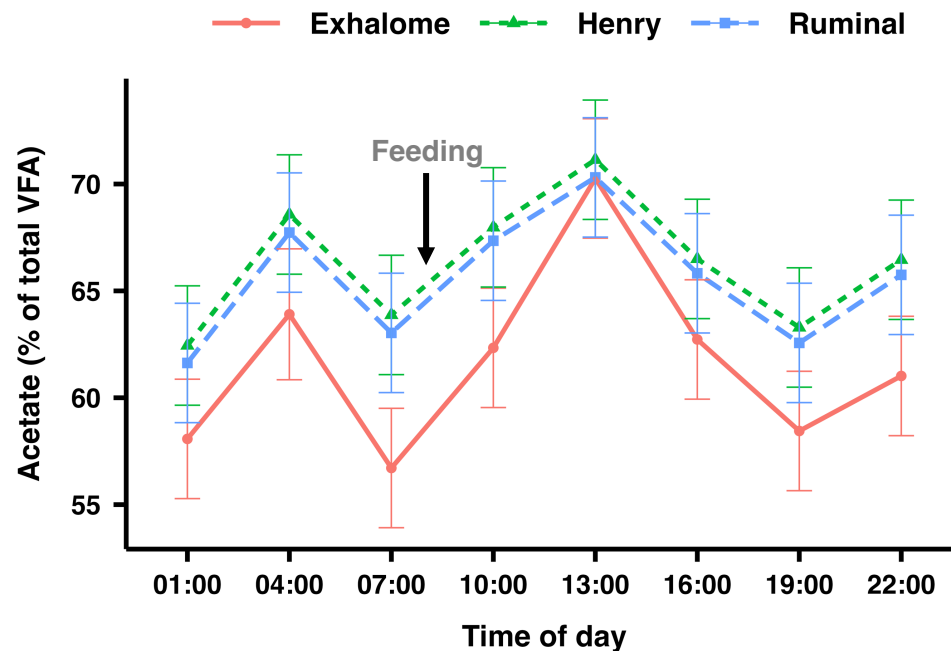
Time, $P < 0.05$
 Method, $P < 0.05$
 Time*Method, $P = 0.88$



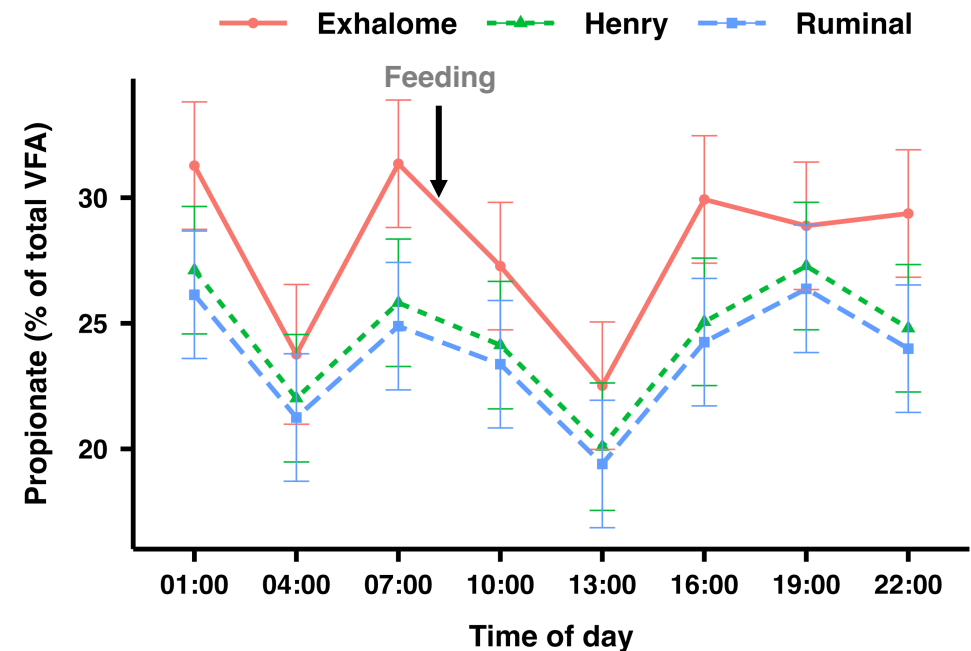
- No method by time interactions

Ruminal vs. Exhaled VFA in 3-h Intervals - Low-starch Diet

Time, $P < 0.05$
 Method, $P < 0.05$
 Time*Method, $P = 0.99$

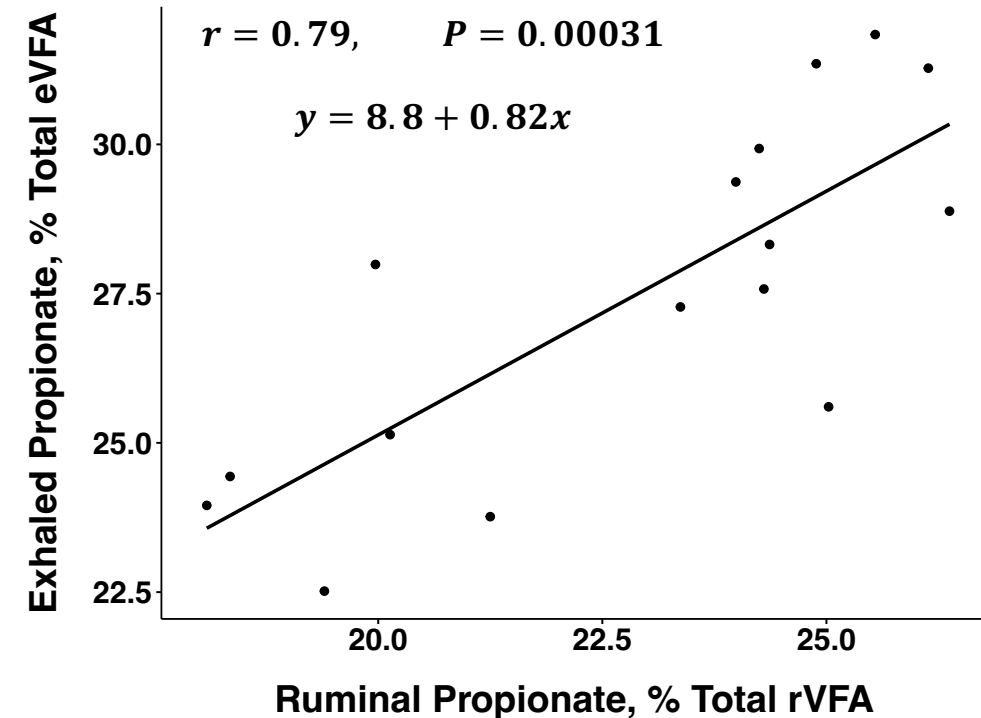
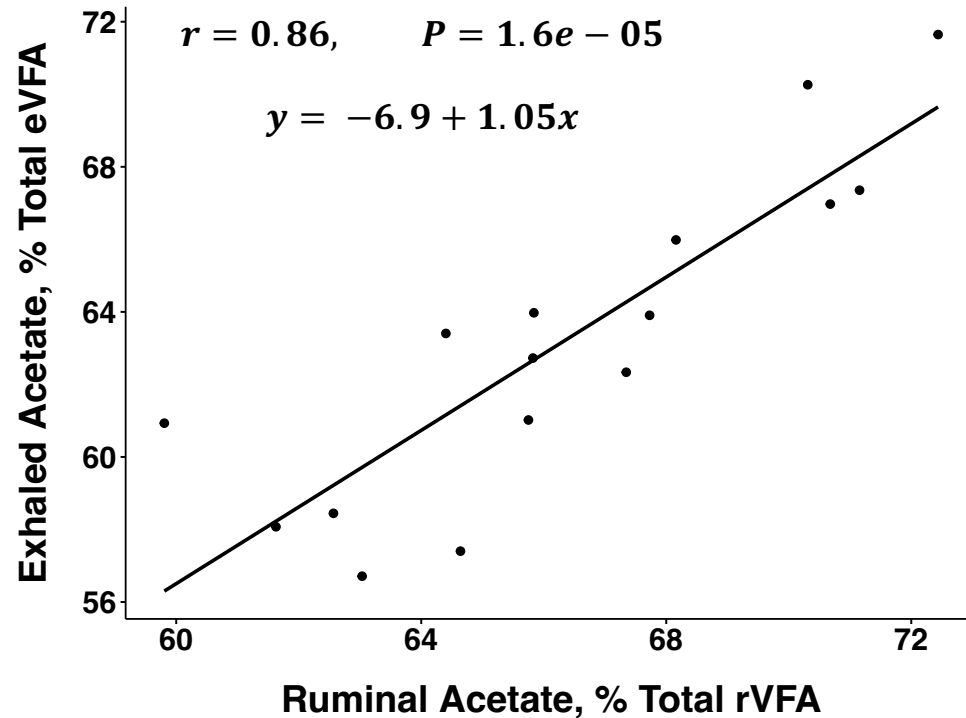


Time, $P < 0.05$
 Method, $P < 0.05$
 Time*Method, $P = 0.99$



- Similar trend in acetate and propionate molar proportions
- The method used may affect the absolute but not relative profile

Linear Regression to Check Mean Bias and Slope Bias



- Strong Pearson correlations, minor slope biases for acetate and propionate

Take-home Message

- Non-invasive exhalomics approach has great potential to monitor and assess rumen fermentation
- Further validation with a larger sample size and adequate statistical power is needed
- Comparison using more commonly used analytical platform is needed (e.g., GC-MS)
- Further exploration of other exhalomics features



Thank You!

Zakirul Islam
zakirul.islam@usys.ethz.ch

