## Can exhaled breath metabolomics replace rumen sampling in dairy cows?

<u>S. Giannoukos<sup>1</sup></u>, Z. Islam<sup>2</sup>, S. Räisänen<sup>2</sup>, A. Schudel<sup>2</sup>, F. Wahl<sup>3</sup>, R. Zenobi<sup>1</sup>, M. Niu<sup>2</sup>

<sup>1</sup>Department of Chemistry and Applied Biosciences, Analytical Chemistry, ETH Zürich, 8093, Zürich, Switzerland, <sup>2</sup>Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, 8092 Zürich, Switzerland, <sup>3</sup>Food Microbial Systems Research Division, Agroscope, 3003 Bern, Switzerland

Previously, we characterized the intensity and daily patterns of exhaled volatile fatty acids (eVFA) using a secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) platform. The aim of this study was to further validate the potential of the exhalomics approach to assess rumen fermentation. Four rumen-cannulated original Swiss (Braunvieh) cows were used in a switchback design with 3, 9-d periods (7-d adaptation, 2-d sampling). Cows were randomly assigned to 1 of 2 diet sequences (ABA/BAB): (A) low-starch (LS; 6.3% starch of DM), and (B) highstarch (HS; 16.2% starch of DM). Feeding was 1×/d at 0800 h. Exhalome (with GreenFeed System) and rumen samples were collected 8x to represent every 3-h of a day, and eVFA and ruminal VFA (rVFA) were analyzed using SESI-HRMS and HPLC, respectively. Data were analyzed in a mixed model with a fixed effect of the period, method, diet, and method×diet interactions, and random effect of time (repeated measures) and cow nested in sequence. Diet×method interactions were not observed. A reduced model was fitted on a method-specific subset of data to test the diet effect. The VFA molar proportions differed between HS vs. LS regardless of method: acetate was 64.1 vs. 60.1 for exhalome (P = 0.01) and 67.0 vs. 64.7 for rumen (P = 0.01), propionate 28.1 vs. 30.5 (P = 0.09) and 22.9 vs. 24.7 (P = 0.04), butyrate 7.87 vs. 9.53 (P = 0.04) and 10.1 vs. 10.7 (P = 0.11); and A:P ratio 2.49 vs. 2.14 (P = 0.05) and 3.13 vs. 2.84 (P = 0.04). For VFA daily patterns, a similar model was fitted for a diet-specific subset of data but with method×time interactions. Regardless of diet, interactions were not observed (P > 0.10). Overall, eVFA and rVFA showed similar responses to feeding and dietary treatments, indicating the potential of eVFA as a proxy to characterize rVFA molar proportions in response to dietary treatments. Future studies should further explore the potential of exhalomics in ruminant research.