

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

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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) high-starch (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.