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SUPPLEMENTAL TABLES AND FIGURES

Exhalomics as a non-invasive method for assessing rumen fermentation in dairy cows: Can exhaled breath metabolomics replace rumen sampling?

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Table S1. Theoretical and measured *m/z* (mass-to-charge) values of exhaled volatile fatty acids detected in negative ion mode at a resolution of 1.4×10^5

Annoted compounds	Theoretical *m/z* $[M-H]^-$	Measured *m/z* $[M-H]^-$	Mass Error[1] (ppm)
Acetate	59.0139	59.0139	< 0.1
Propionate	73.0295	73.0296	1.8
Butyrate[2]	87.0452	87.0453	1.1
Valerate[3]	101.0602	101.0605	3.0

[1]Formula used to calculate the mass error for the characterized VFA features:

$$\text{Mass Error (ppm)} = \left(\frac{\text{Measured m/z} - \text{Theoretical m/z}}{\text{Theoretical m/z}}\right) \times 10^6$$

[2]Butyrate = exhaled butyrate + exhaled isobutyrate, because the isomers have identical *m/z* values as the relative compound butyrate.

[3]Valerate = exhaled valerate + exhaled isovalerate, was considered as a sum due to the same *m/z* values as the relative compound valerate.

Table S2. Summary statistics of the raw concentrations and molar proportions of volatile fatty acids measured in three different methods

	Ruminal (n = 96)					Henry's Law (n = 96)					Exhaled (n = 96)				
Items	Mean	Median	Min	Max	SD	Mean	Median	Min	Max	SD	Mean	Median	Min	Max	SD
VFA Concentrations[1]															
Acetate	65.5	66.1	53.4	73.9	6.03	0.353	0.357	0.288	0.400	0.0326	27.6	26.6	10.0	42.3	8.73
Propionate	24.8	24.3	12.5	36.4	6.54	0.143	0.141	0.072	0.211	0.0378	12.8	12.8	4.59	22.0	4.19
Butyrate[2]	11.1	9.89	7.67	22.3	3.13	0.042	0.037	0.029	0.083	0.0117	3.93	3.71	1.73	6.95	1.235
Isobutyrate[2]	2.47	2.38	0.89	4.60	0.966	0.009	0.009	0.003	0.017	0.0036	-	-	-	-	-
Valerate[3]	0.72	0.65	0.13	1.76	0.381	0.003	0.003	0.002	0.008	0.0018	2.70	2.65	0.92	4.52	0.860
Isovalerate[3]	1.36	1.29	0.67	2.70	0.508	0.006	0.005	0.003	0.011	0.0022	-	-	-	-	-
Total VFA	106	106	78.0	135	12.80	0.556	0.558	0.408	0.703	0.0668	47.0	48.0	19.3	68.3	13.21
VFA, mol %															
Acetate	62.4	62.5	52.4	71.1	4.89	64.1	64.1	53.9	73.1	4.97	58.2	59.0	44.0	70.0	5.60
Propionate	23.0	23.2	15.8	31.9	4.23	25.3	25.6	17.4	34.8	4.59	27.2	26.9	17.8	36.6	4.34
Butyrate[2]	12.7	12.0	9.86	21.9	2.405	9.02	8.52	6.92	16.2	1.812	8.84	8.82	4.25	15.6	2.818
Valerate[3]	1.96	1.95	1.16	3.31	0.537	1.63	1.61	0.94	2.79	0.455	5.74	5.69	3.55	7.58	0.959
A:P[4]	2.92	2.72	1.73	4.58	0.788	2.72	2.54	1.62	4.28	0.735	2.40	2.29	1.34	4.58	0.704

[1]m*M* = for ruminal and Henry's Law, and CPS = count-per-second for exhaled (instrumental signal intensity of each VFA measured in SESI-MS, represents relative concentration).
[2]Butyrate = exhaled butyrate + exhaled isobutyrate, since isomers have identical *m/z* values as the relative compound butyrate.
[3]Valerate = exhaled valerate + exhaled isovalerate, was similarly considered as a sum due to the same *m/z* values as the relative compound valerate.
[4]A:P = Acetate-to-Propionate ratio.

Table S3. Molar proportions of volatile fatty acids measured in 3-h intervals from dairy cows fed either high-starch or low-starch diets utilizing three distinct method

	Ruminal								**Henry's Law**								**Exhaled**								*P*-value			
	Time of day, h[1]								Time of day, h								Time of day, h											
VFA, mol %	0100	0400	0700	1000	1300	1600	1900	2200	0100	0400	0700	1000	1300	1600	1900	2200	0100	0400	0700	1000	1300	1600	1900	2200	SE[2]	M[3]	T[4]	M x T
High-starch diet																												
Acetate	61.6	61.8	57.6	69.6	68.2	67.6	65.6	63.6	63.3	63.1	59.6	71.3	70.1	69.2	67.6	64.9	60.0	53.9	57.7	68.2	64.0	63.7	62.5	60.5	2.16	<0.01	<0.01	0.92
Propionate	23.2	24.4	24.0	17.4	17.6	19.3	19.2	23.6	25.7	26.8	26.8	19.2	19.4	21.2	21.3	25.8	26.0	29.7	24.2	22.7	23.2	23.8	26.4	29.0	1.99	<0.01	<0.01	0.81
Butyrate	13.3	11.5	14.0	10.9	12.3	10.6	12.4	10.7	9.46	8.13	10.0	7.74	8.82	7.51	9.73	7.56	8.26	8.55	10.7	3.94	7.55	7.26	5.47	5.97	0.806	<0.01	<0.01	0.14
A:P	2.69	2.67	2.49	4.03	3.87	3.42	3.58	2.85	2.52	2.50	2.33	3.77	3.62	3.20	3.35	2.67	2.48	1.96	2.23	3.16	2.84	2.78	2.55	2.05	0.297	<0.01	<0.01	0.93
Low-starch diet																												
Acetate	58.9	65.0	61.0	64.5	67.3	63.6	60.1	63.0	60.7	66.8	62.7	66.0	69.2	65.0	61.7	64.5	50.0	60.9	53.4	54.3	71.8	59.2	55.4	57.8	2.56	<0.01	<0.01	0.42
Propionate	24.7	20.3	23.9	22.2	18.4	23.3	25.1	22.8	27.4	22.4	26.4	24.4	20.4	25.6	27.7	25.1	32.3	22.4	29.2	25.5	24.5	28.0	27.0	27.4	2.88	<0.01	<0.01	0.99
Butyrate	14.3	12.2	13.6	10.9	12.3	11.0	13.4	11.8	10.2	8.69	9.67	7.69	8.71	7.76	9.54	8.36	9.76	8.27	11.0	9.59	6.68	6.72	6.86	6.88	1.06	<0.01	<0.01	0.74
A:P	2.52	3.25	2.62	3.26	3.73	3.08	2.32	2.85	2.36	3.05	2.46	3.05	3.49	2.88	2.54	2.67	1.59	2.53	1.96	2.07	3.25	2.22	2.27	2.42	0.305	<0.01	<0.01	0.95

[1]Time of day indicates the actual time of sampling across three methods.
[2]SE = Standard error.
[3]M = VFA measurement methods.
[4]T = Time of day.

Figure S1. Intra-day pattern of A. feed intake rate, %/h, B. starch intake, kg/h, and C. NDF intake, kg/ h in dairy cows fed a high and low-starch diet. The gray dashed lines indicate feeding time (0830 h). Feed intake from 0700 to 0800 is omitted due to feed refusal cleaning and fresh feed delivery.

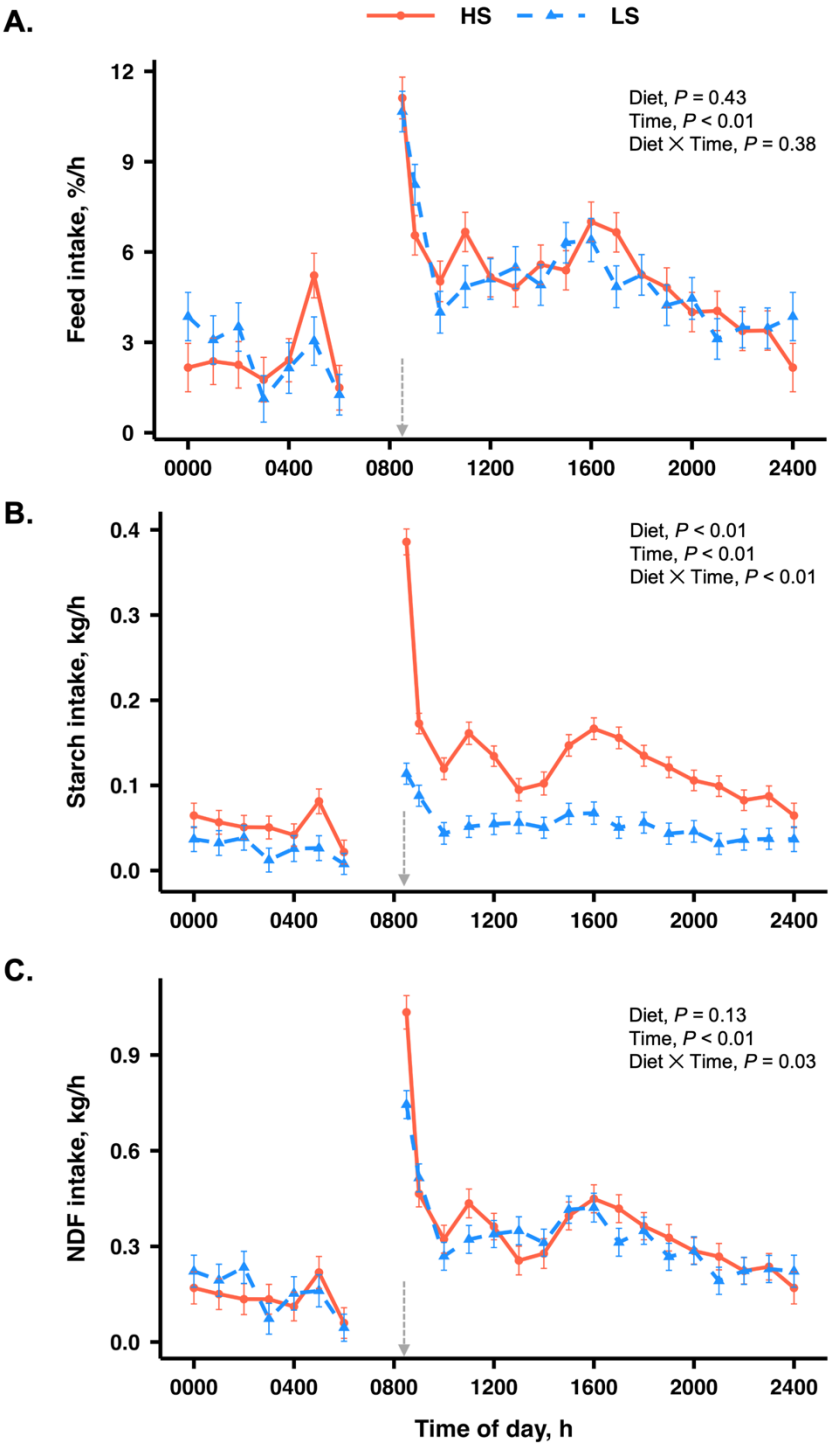

Figure S2. Rumen pH (A) and temperature (B) of dairy cows fed high-starch (HS) and low-starch (LS) diets measured in 3-h intervals (LSM ± SE)

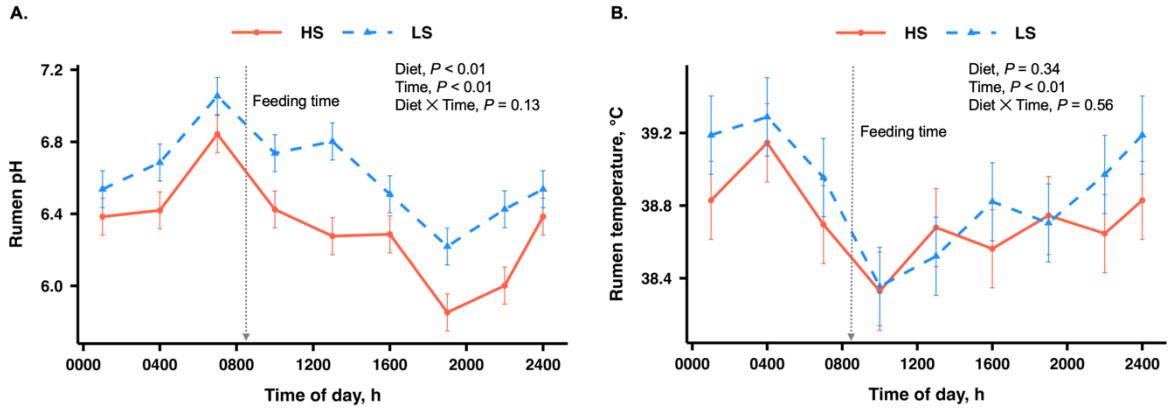

Figure S3. Correlation between the molar proportions of Henry's Law VFA and Exhaled VFA, (A) acetate, (B) propionate, (C) acetate-to-propionate ratio, and (D) butyrate using LSM from mixed model (ii) outputs. The significant interaction term between diet (HS = High-starch, and LS = Low-starch) and exhaled VFA is depicted by two fitted lines for acetate (Diet × EX-VFA, $P = 0.05$) with no interaction confirmed by a common slope across both diets (green fitted line) for propionate ($P = 0.60$), acetate-to-propionate ratio ($P = 0.12$), and butyrate ($P = 0.91$). The black dashed line represents the identity relationship (y = x).