

Supplemental Materials

Table S1. Test Day Least Square Means (LSM) for production, head chamber yield, gas dry matter intake with a correction factor (cDMI) yield, and intensity of methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), and ammonia (NH₃) from all Holstein dairy cattle enrolled in the trial (n = 20).

	Test Day LSM					SEM	<i>p-values</i> Day
	Day 0 ¹	Day 14	Day 28	Day 42	Day 56		
Production							
CH ₄ (g/period)	399	338 ^a	390 ^b	367 ^b	380 ^b	11.4	<.001
CO ₂ (g/period)	9800	8747 ^a	9990 ^b	9392 ^{ac}	9688 ^c	267	<.001
N ₂ O (mg/period)	1705	1339	1379	1295	1332	43.3	0.32
NH ₃ (mg/period)	423	331 ^a	358 ^a	285 ^b	274 ^b	11.7	<.001
Head Chamber Yield²							
CH ₄ (g/period/kg)	26.5	23.8	25.3	23.6	24.6	0.65	0.17
CO ₂ (g/period/kg)	652	620	652	608	630.7	19.1	0.18
N ₂ O (mg/period/kg)	113	94.0 ^a	89.8 ^{ab}	83.3 ^b	86.4 ^{ab}	2.66	0.012
NH ₃ (mg/period/kg)	28.0	23.7 ^a	23.3 ^a	18.6 ^b	17.9 ^b	0.97	<.001
cDMI Yield³							
CH ₄ (g/period/kg)	19.6	16.9 ^a	18.5 ^b	17.5 ^{ab}	18.2 ^b	0.45	0.005
CO ₂ (g/period/kg)	481	438 ^a	475 ^b	449 ^{ab}	467 ^b	8.80	0.003
N ₂ O (mg/period/kg)	83.4	66.6	65.4	61.6	64.0	1.42	0.09
NH ₃ (mg/period/kg)	20.7	16.8 ^a	16.6 ^a	13.6 ^b	13.2 ^b	0.44	<.001
Intensity⁴							
CH ₄ (g/period/kg)	9.43	7.72 ^a	9.16 ^b	9.13 ^{ab}	9.72 ^b	0.39	<.001
CO ₂ (g/period/kg)	107	202 ^a	236 ^b	235 ^b	249 ^b	10.3	<.001
N ₂ O (mg/period/kg)	18.5	30.8	32.5	32.3	32.9	1.31	0.36
NH ₃ (mg/period/kg)	4.60	7.71 ^{ab}	8.62 ^a	7.10 ^b	7.01 ^b	0.37	<.001

Within rows, means with different superscript differ ($p < 0.05$). Differences between means determined by Tukey's multiple comparison test.

Period = 12-hour gas emission sampling period

¹Day 0 included as covariate within the model for the four test days (when treatment was applied)

²gas production × (1/kg DMI from the sampling period while in the HC)

³gas production × (1/kg corrected DMI) according to Robinson et al. [29]

⁴ gas production × (1/kg energy corrected milk)

Table S2. Test Day Least Square Means (LSM) for feed efficiency, daily dry matter intake (DMI), energy corrected milk (ECM), Milk fat, milk protein, milk urea nitrogen (MUN), and serum urea nitrogen (SUN) from all Holstein dairy cattle enrolled in the trial (n = 20).

	Test Day LSM					SEM	<i>p-values</i>
	Day 0 ¹	Day 14	Day 28	Day 42	Day 56		Day
Feed Efficiency ²	1.64	1.69 ^a	1.61 ^b	1.56 ^{bc}	1.54 ^c	0.03	<.001
DMI (kg)	25.9	26.1 ^a	26.7 ^b	26.4 ^a	26.0 ^a	0.24	0.003
ECM (kg)	42.4	43.6 ^a	42.5 ^a	40.7 ^b	39.6 ^b	0.85	<.001
Milk Fat (kg)	1.71	1.81 ^a	1.71 ^b	1.61 ^c	1.56 ^c	0.04	<.001
Milk Protein (kg)	1.11	1.10 ^a	1.14 ^b	1.12 ^{ab}	1.12 ^{ab}	0.02	0.002
MUN (mg/dL)	10.1	10.9 ^a	9.20 ^b	9.23 ^b	9.38 ^b	0.30	<.001
SUN (mg/dL) ³	11.7	12.5 ^a	12.3 ^a	10.9 ^b	11.9 ^a	0.36	<.001

Within rows, means with different superscript differ ($p < 0.05$). Differences between means determined by Tukey's multiple comparison test.

¹ Day 0 included as covariate within the model for the four test days (when treatment was applied)

² kg ECM/kg daily DMI

³ Samples were collected following morning milking session (hour 0)