in triplicate. CH_4 production was analyzed by gas chromatography. The copy numbers of the 16S rRNA gene of methanogenic archaea were quantified using RT-PCR. We used a fixed effects model and analyzed the data using the PROC GLM procedure of the SAS® software. A post hoc analysis was performed for the dose-by-hour interaction using the lsmeans procedure and the Scheffé adjustment for pairwise comparisons. We detected a dose-by-hour interaction for CH_4 production ($P = 0.001$) and pH ($P = 0.03$), whereas for total gas, a main effect of hour ($P < 0.0001$) was found. The lowest CH_4 production (0.46 ± 5.38 mL) was found with the 16 m*M* dose at 6 h post-incubation, matching the lowest gas production (135 ± 7.47 mL) observed at the same dose and hour. We also observed that the copy numbers of methanogenic archaea were affected by hour ($P < 0.0001$) and dose ($P < 0.0001$), which were lowest (6.77 ± 0.15) with the 8 m*M* dose at 6 h post-incubation, suggesting a rapid suppressing effect. CH_4 production, total gas, and copy numbers of methanogenic archaea were at their lowest and highest points at 6 and 24 h post-incubation, respectively, suggesting that ENA was being metabolized as the incubation progressed. In summary, these findings indicate that the antimethanogenic effect of ENA is potentiated by dose and hour and that they act synergistically to reduce CH_4 production by suppressing the metabolism of methanogenic archaea.

Key Words: nitro-acetate, methane production, methanogenic archaea

1334 Monensin reduces enteric methane emissions and intensity in late-lactation Holstein cows consuming a high-concentrate diet. D. Onan-Martinez*[1], M. A. T. de Bari[1], H. Olmo[1], J. Lance[1], I. M. Toledo[1], J. M. Tricarico[2], and G. E. Dahl[1], *[1]Department of Animal Sciences, University of Florida, Gainesville, FL, [2]Dairy Management Inc., Rosemont, IL.*

Methane (CH_4) is a major greenhouse gas responsible for global warming. Multiple strategies have been tested to reduce enteric methane yield from cattle. Among them, supplementation with monensin has shown variable outcomes. However, we hypothesized that Rumensin (monensin) would reduce enteric CH_4 yield and intensity in late-lactation dairy cows on a high-concentrate diet (~207 DIM). Cows (n = 20) were fed a high-concentrate diet (51.3% grain mix and 48.7% forage on DM basis) during two 4-week periods in a crossover design with 2 treatments: control (CON) and monensin (MON). Statistical analysis by Proc Mixed used a model that included treatment, period, order, time, and the interactions as fixed effects. MON consisted of 300 mg/cow of monensin in 34 g/cow of dried distillers grains (DDG), whereas CON was DDG alone, both top-dressed daily on the TMR. All cows were milked 3 times daily and housed in a sand-bedded freestall barn equipped with Calan gates to collect individual dry matter intake (DMI). A GreenFeed system was used to measure enteric gas output. Daily milk yield and DMI were measured from each cow for the entire study. In addition, milk fat, protein, and lactose were measured weekly. Average DMI (MON = 25.4 ± 0.3, CON = 25.4 ± 0.3, kg/d ± SEM; $P > 0.1$) and energy-corrected milk (ECM) yield (MON = 35.8 ± 1.09, CON = 36.4 ± 1.09, kg/d ± SEM; $P > 0.1$) were similar between treatments. Conversely, daily methane emissions were significantly reduced ($P < 0.01$) with Rumensin (MON = 207.1 ± 13.1, CON = 257.2 ± 13.1 g/d). Methane yield also declined ($P < 0.05$) with treatment (MON = 8.1 ± 0.4, CON = 9.9 ± 0.4 g/kg DMI). Lastly, methane intensity relative to ECM tended to decline ($P = 0.08$) with Rumensin (TRT = 5.7 ± 0.3, CON = 6.5 ± 0.3 g/kg of ECM). The results suggest that Rumensin reduces enteric methane emissions and intensity in late-lactation dairy cattle fed high-concentrate diets, providing evidence to support the use of monensin to reduce CH_4 emissions from cattle.

Key Words: dairy cattle, GreenFeed, enteric gas output

1335 Feed intake regulation stabilized daily intake pattern but reduced lactational performance with little impact on rumen fermentation or efficacy of 3-nitrooxypropanol (3-NOP) in dairy cows. K. Wang*[1], S. E. Räisänen[1], L. Eggerschwiler[2], T. He[1], M. Z. Islam[1], Y. Li[1], X. Ma[1], R. Siegenthaler[2], Z. Zeng[1], F. Dohme-Meier[2], and M. Niu[1], *[1]Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH, Zürich, Switzerland, [2]Ruminant Nutrition and Emissions, Agroscope, Posieux, Switzerland.*

To study the effect of feed intake regulation (i.e., stabilizing intake fluctuations) on rumen fermentation and CH_4 emissions, 16 multiparous Holstein cows (126 ± 19.7 DIM, 34 ± 3.8 kg/d milk yield) were used in a 4 × 4 Latin Square design with four 21-d periods (14-d adaptation, 7-d sampling). Four treatments consisted of a 2 × 2 factorial arrangement of feed access levels (ad libitum [AL] vs. regulated [RE]) and 3-NOP levels (no vs. yes; 70 mg/kg DM). All cows were housed in a freestall barn, and TMR (14% CP and 42% NDF of DM) was delivered 2×/d at 0700 and 1700 h. The RE access to feed was programmed using the Insentec (Hokofarm Group B.V.) system as follows: a cow was allowed to consume up to 12% of the daily feed intake (based on the previous week's intake) in each meal; once the limit was reached, the cow was denied access to the feed trough for 1 h. Data were analyzed using linear mixed model in R. There was a feed access by time interaction ($P < 0.01$) on daily pattern of feed intake (FI): FI rate (% of DMI) was reduced ($P < 0.01$) for RE vs. AL at 2 conditional meals after feeding (5.7 vs. 9.0%/h and 6.1 vs. 12.7%/h, respectively) and was compensated for cows with RE access during night (4.2 vs. 3.1%/h from 1900 to 2400 h, $P < 0.01$). Cows with RE had a lower intraday variation (1.7 vs. 2.8%/h of SD) in FI compared with AL. There were no interactions between feed access and 3-NOP on production, rumen VFA, and CH_4 emission parameters. Cows with RE access had a lower ($P < 0.05$) DMI (−0.37 kg/d), milk yield (−1.04 kg/d), and ECM (−1.17 kg/d) but showed no difference in VFA and CH_4 production. The molar proportions (mol%) of acetate (−3.24) decreased, but propionate (0.93) and butyrate (2.56) increased in cows supplemented with 3-NOP ($P < 0.05$). Methane production (g/d), yield (g/kg DMI), and intensity (g/kg ECM) were reduced ($P < 0.05$) by 3-NOP by 33%, 32%, and 30%, respectively. In conclusion, the stabilized intake pattern did not further affect rumen VFA, CH_4 production, and mitigating efficacy of 3-NOP in the current study.

Key Words: intake regulation, rumen fermentation, methane emission

1336 Effect of different doses of 3-NOP combined with varying forage composition on methane yield, feed intake, and milk production in dairy cows. M. Maigaard*[1], M. R. Weisbjerg[1], C. Ohlsson[2], N. Walker[2], and P. Lund[1], *[1]Department of Animal and Veterinary Sciences, AU Viborg–Research Centre Foulum, Aarhus University, Tjele, Denmark, [2]DSM-Firmenich, Kaiseraugst, Switzerland.*

The objective of this study was to investigate the effect of different doses of 3-nitrooxypropanol (3-NOP) with varying forage composition on gas emission and production performance of dairy cows. Seventy-two lactating Danish Holstein cows were enrolled in a continuous randomized block design with 2 weeks of adaptation, after which treatments were applied for 12 consecutive weeks. Initial DMI and ECM yield were 24 ± 3.3 and 38 ± 7.1 kg, respectively. Cows were blocked according to parity and DIM. Treatments were organized in a 2 × 3 factorial arrangement. The first factor was diet type, reflecting 2 different forage compositions of the diet. Ratio of grass-clover silage to corn silage was 60:40% of total forage DM in grass-based diets and 40:60% in corn-based diets. Total forage constituted 56% of the DM in the partial mixed rations. The second factor reflected 3 doses of 3-NOP: 0, 60, or 80 mg 3-NOP/kg DM. Gas emission were measured using GreenFeeds and feed intake