

## Evaluation of compound<sup>#1</sup> supplementation levels on *in vitro* rumen fermentation characteristics and methane emission

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This study evaluated the effects of compound<sup>#1</sup>, a candidate compound for methane mitigation, on rumen fermentation characteristics and methane production using an *in vitro* system. Rumen fluid was collected from three Holstein dairy cows using a stomach tube and mixed with McDougall's buffer at a 1:4 ratio. A 50 mL aliquot of this mixture was anaerobically dispensed into 125 mL serum bottles containing 500 mg (dry matter basis) of a total mixed ration (TMR) substrate, supplemented with compound<sup>#1</sup> at concentrations of 0, 240, 480, 720, and 2,000 ppm (based on TMR substrate). The experiment was conducted following a completely randomized design with three replicates per treatment and four incubation times (0, 12, 24, and 48 hours), resulting in a total of 60 bottles. Dry matter degradability and total volatile fatty acid (VFA) concentrations were not significantly affected by compound<sup>#1</sup> supplementation ( $p > 0.05$ ). However, supplementation at 480 ppm or higher significantly reduced methane production and increased propionate concentrations after 12 and 24 hours of incubation ( $p < 0.05$ ). However, acetate concentrations were significantly decreased at 720 and 2,000 ppm after 12 and 24 hours ( $p < 0.05$ ). These results suggest that supplementation with compound<sup>#1</sup> at 480 ppm can effectively reduce methane emissions without adversely affecting rumen fermentation or productivity in Holstein dairy cows.

**Key words :** compound<sup>#1</sup>, *in vitro*, methane emission, volatile fatty acids