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Session 5a) Novel Methodology 1: Application of Isotopologue Measurement

Opening isotopic windows into the rumen microbiome with Orbitrap MS

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Ruminant animals (e.g. cows) are a major source of the potent greenhouse gas methane, but they are also a tractable target for climate solutions. Multiple strategies have been developed to lower methane emissions from ruminants, including feed additives that inhibit the resident population of methanogenic microbes. To work as a sustainable methane reduction strategy, these additives must eliminate methane emissions without hampering the microbial fermentation of plant material. Fermenting bacteria generate organic acids (e.g. acetate, propionate and butyrate) for the animal host to use as its primary source of carbon and energy. As such, fermentation is an essential process to maintain in the rumen. However, we currently lack the biological tools to quantify how carbon flow through rumen fermentation is altered when methanogens are inhibited by chemical additives. Here, we developed a rapid method to measure the compound-specific stable isotope ratios ($^{13}C/^{12}C$ and $^{2}H/^{1}H$) of acetate, propionate and butyrate simultaneously using an electrospray (ESI) Orbitrap mass spectrometry (MS) that could quantify in vivo shifts in fermentation pathways. We investigated the myriad sources of analytical matrix effects, including ionization suppression and mass spectral interferences, to confirm the fidelity of this method. We then applied the ESI-Orbitrap technique to incubations of rumen fluid with and without the additive *Asparagopsis taxiforms,* a red macroalgae that inhibits methanogens. We found that the isotope composition of VFAs changed between these conditions, reflecting a metabolic shift in the fermentation of plant material. Our study demonstrates that ESI-Orbitrap MS could be a useful method for evaluating the efficacy and sustainability of engineered methane mitigating strategies in bovine rumen. It may also be a promising tool for studying animal gut microbiome ecology more broadly.