Effects of chemotaxis on ruminal protozoa when treated with nitrate and nitrite

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Rumen microorganisms in cattle degrade feed and ferment the resultant sugars to end products that contribute to 50-80% of the animal's metabolizable energy. Methane, a possible end product of fermentation, is 23 times more potent of a greenhouse gas than CO2. Decreasing enteric methane production, while still maintaining productivity, is a priority among the agricultural industry. Nitrate (NO3) is an effective methane mitigator because it is thermodynamically more favorable for bacteria to utilize excess H2 by chemically reducing NO3 to NO2 and then to NH3 than it is for methanogens to use H2 to reduce CO2 to CH4. It is not understood how NO3 or NO2 affect the chemotaxis of protozoa because they might have activity for NO-generated circular swimming. Based off work by Diaz, pre-incubation with sodium nitroprusside (SNP; an NO generator) increased chemotaxis (mechanism to help locate food) toward glucose for entodiniomorphids. In contrast, we do not know if excessive stimulation is toxicWe hypothesized that treatment with NO3 and NO2 will increase chemotaxis (and circular swimming) similar to results observed in protozoa treated with SNP and will decrease overall motility due to toxicity at the higher concentrations of NO3 and NO2.Rumen fluid was collected from two cannulated Jersey cows, flocculated, and inoculated into beakers (25mL) dosed with either 0.9% saline (-control), 500 □M SNP (+control), 5.68mM (1x) of NO2, (1x) NO3, 11.36mM (2x) NO2, or (2x) NO3. After 3h, samples were taken for subsequent protozoal counting and videography for motility analysis. Capillary tubes filled with a nutrient source (glucose or peptide) or saline (control) were positioned inside the remaining fluid and incubated for 20 min. Capillary tube contents were preserved and internalized protozoa were counted to assess the effect on chemotaxis. Two replications were done per day, and the experiment was repeated over three days.