Determining the effect of protozoal inhibitors on protozoal motility to improve ruminant feed efficiency and reduce enteric methane

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Enteric methane production is estimated to contribute 17% of global methane, produced exclusively by a group of Archaea known as methanogens, which tend to associate with protozoa as a symbiotic source of substrate (formate or CO2 and H2). Recent studies have focused on direct inhibition of methanogens or decreasing the availability of H2 or formate. Monensin (MON), an antibiotic not used in human therapy, is a feed additive used to improve production efficiency in cattle. Essential oils are non-antibiotic alternatives that selectively inhibit groups of microbes and include products such as Cinnagar® (CIN), a combination of cinnamon and garlic essential oils. This study used 240 videos during an in vitro trial to examine the effects of MON and CIN treatment on protozoal motility. Previous research on these additives decreased an indirect measurement of protozoal volume but had variable consequences for N/cell ratio. We hypothesized that MON and CIN would have additive inhibitory effects on protozoal function and motility. Protozoa were given a control treatment (CON), or treatment with MON, CIN, or MON+CIN in a 2 x 2 factorial arrangement of treatments. Replicate tubes at 3 hours post-feeding were analyzed for distance, average speed, and average protozoal area with ImageJ software and using hour 0 as a covariate. The main effect of MON decreased protozoal distance by 134 um and average speed by 66.2 um/s ($P<0.05$). The main effect of CIN decreased average area by 256 um2 ($P<0.05$). MON interacted ($P<0.05$) with CIN for average speed, demonstrating CIN decreased MON's inhibition; simple treatment means for CON, MON, CIN, and MON+CIN were 243, 138, 211, and 183 um/s, respectively. Compared with more tedious and time-consuming protozoal motility methods, the current method will improve efficiency, accuracy, and/or precision for future studies assessing the role of protozoal ecology on enteric methane production in cattle.