

Abstract

The bovine rumen harbors a complex microbial community composed of a large number of species, whose importance to the cow and impact on the environment are reflected in many ways. These include specialization in the efficient breakdown and digestion of plant-based feed, effects on cow health, and the emission of waste products and gases into the environment. Despite recent advances in understanding the complexity of the processes taking place in the rumen, and technological developments that have enabled a deeper view of this microbial community, the role of some rumen microbial groups remains unclear. This gap is particularly evident for protozoa, a group of eukaryotic cells that constitute approximately 50% of the microbial biomass in the rumen.

Based on preliminary results from our laboratory, in which we characterized microbiome composition under different diets, we observed that, in contrast to the bacterial community, whose composition is strongly affected by diet, the protozoal community is more stable, yet differs substantially between individual cows. This observation suggests that additional forces, beyond environmental conditions such as those induced by diet, shape the composition of the protozoal community.

Therefore, in this study, we aimed to test the hypothesis that the protozoal community is determined by initial acquisition and remains stable thereafter, in contrast to the bacterial community, which changes in response to diet.

In this study, together with samples derived from previous studies, we collected rumen fluid from 50 heifers from birth until first milking. This represents an unprecedented dataset in Israel, enabling us to examine our hypotheses.

Analysis of the protozoal and bacterial communities over time showed that protozoal communities among different heifers were more similar to one another early in life than at later developmental stages. This stands in complete contrast to bacterial communities, for which both this study and previous studies show an increase in similarity among individuals over time. This finding indicates that the assembly process of the protozoal community is fundamentally different from that of the bacterial community.

In addition, we measured methane production and quantified the relative contribution of the protozoal community on an individual basis in adult cows. These results showed that different cows, harboring different protozoal communities, exhibit substantial

variation in the contribution of protozoa to methane production, ranging from a 5% contribution to as much as an 80% increase in methane production.

We then quantified the volatile fatty acid profile and examined the relationship between changes in methane production and changes in VFAs following the addition of the protozoal community. These results showed that, in contrast to the consistent increase in methane production, the effect of protozoa on the VFA profile was more moderate and did not indicate a consistent shift across all cows, although certain positive associations were observed between the increase in methane and changes in acetate and butyrate.

Overall, the findings of this study indicate that the rumen protozoal community is not merely a stable component whose composition differs between cows, but also a functional factor with an individual and variable contribution to methane production. These findings may contribute to a better understanding of the microbial factors influencing methane emissions in cows and may, in the future, open the possibility for early identification or guidance of protozoal communities with lower contributions to methanogenesis, as part of strategies to improve production efficiency and reduce the environmental impact of the dairy industry.