Proc. Assoc. Advmt. Anim. Breed. Genet. 26: 245-248

EVALUATING ORAL SWABBING AS A PRACTICAL ALTERNATIVE TO RUMEN SAMPLING FOR LARGE-SCALE METHANE EMISSION STUDIES IN GRAZING ANIMALS

C.T. Ong[1], Z. Chen[1], A.S. Cavallaro[1], M.R.S. Fortes[1,2], M. Dekker[2], A. Boulton[2], B. Kniwesky[2] and E.M. Ross[1]

[1] The University of Queensland, QAAFI, Brisbane, QLD, 4072 Australia
[2] The University of Queensland, SCMB, Brisbane, QLD, 4072 Australia

SUMMARY

The oral microbiome has been proposed as a proxy for the rumen microbiome. This study aimed to evaluate this approach in grazing cattle. Results from 18 cows revealed significant differences in the diversity and composition of oral and rumen microbiomes. However, methanogen profiles were highly similar between the two sample types. These findings indicate that oral samples can effectively capture key aspects of the rumen microbiome for methane studies due to their relationship to rumination behaviour. This study highlights a practical approach for using the analysis of the oral microbiome analysis as a proxy for rumen microbiome in methane emission research in grazing animals.

INTRODUCTION

The rumen microbiome has been explored from various angles in recent years, to evaluate its involvement in ruminant production, disease resistance and environment sustainability. Nonetheless, rumen content collection poses a significant challenge to leveraging the potential of rumen microbiome data. Common methods, such as ruminal fistulation, stomach tubing, or rumenocentesis, are invasive and contrary to animal welfare principles. Moreover, these methods are labour-intensive, increasing the time cattle must remain restrained in a crush and elevating their stress levels. As a result, rumen collection is impractical and unsuitable for large-scale deployment or commercial farm settings. To address this, our study aims to evaluate the potential of using the oral microbiome as a proxy for the rumen microbiome in grazing cattle.

MATERIALS AND METHODS

Samples were collected from 18 non-pregnant Brahman cows grazing at the University of Queensland's (UQ) Gatton pasture. Oral and rumen microbiome samples were taken at three timepoints: Winter (August 2023), Spring (November 2023), and Summer (January 2024). The same cattle were sampled, for both oral and rumen, on each occasion within 5 minutes of entering the crush. All collections occurred between 10 a.m. and 12 p.m. Animal procedures were approved under Animal Ethics Approval AE000438 by the UQ Animal Ethics Committee. In total, 54 oral and 54 rumen samples were collected.

Each sample was vortexed for 15 seconds and centrifuged at 12,000 x *g* to pellet the contents. DNA was extracted using the QIAGEN QIAamp PowerFecal Pro DNA Kit (QIAGEN, Germany) following the manufacturer's instructions. To obtain microbiome signatures, oral and rumen samples were prepared using the ONT Native Barcoding SQK-NBD114.96 kit (ONT, UK). The ligated DNA library was loaded onto FLO-PRO114M PromethION flowcells and sequenced using the ONT PromethION 2 Solo sequencer with MinKNOW software version 24.06.10. Sequencing continued until each sample reached 1 million reads. The raw data was rebase-called using Dorado v0.6.2.

Base-called reads were assessed using NanoPlot 1.3.0. To remove bovine reads, they were mapped to the ARS-UCD1.2 *Bos taurus* genome (GCA_002263795.2) and Brahman genome (Ross *et al.* 2022) using Minimap2 v2.17, and unmapped (non-host) reads were retained. Chopper v0.9.0

trimmed the first 15 bases of non-host reads and selected those longer than 100 bp with a quality score above 7. Each sample was then subsampled to 1.5 million reads using seqtk v1.4. Taxonomic and functional assignments were conducted using SqueezeMeta v1.6.5, performing Diamond BlastX against the nr, COG, and KEGG databases. Non-microbial sequences, such as viruses, animal, and plant, were excluded. Bioinformatic analysis and visualisation were carried out in R Studio using the *phyloseq* v1.42, *vegan* v2.6.6.1, *DeSeq2* v1.38.3, and *ggplot2* v3.5.1 packages.

RESULTS AND DISCUSSION

To determine the potential of using oral microbiome as a proxy for rumen microbiome in grazing cattle, both samples were collected from 18 empty Brahman cows across three seasons. The sequencing results revealed the high host contamination in oral samples, which constituted an average of 81.28% cattle data in the raw sequencing data (Table 1). This observation was consistent with the high host contamination observed in oral samples, including those from other organisms (Kumar *et al.* 2023).

Table 1. The statistics of sequencing data after each step of data processing

	Rumen				Oral			
	Raw	Nonhost	Chopper	Subset	Raw	Nonhost	Chopper	Subset
Mean Read N50	3,888.39	3,873.32	2,032.34	2,032.32	1,670.22	1,977.41	2,426.60	2,426.72
Mean Quality	15.92	15.92	16.85	16.85	13.18	10.24	12.91	12.91
Mean Reads # (K)	2,241.23	2,218.24	1,736.63	1,362.21	2,285.38	439.86	330.60	321.03
Mean Total Bases (Mb)	3,063.40	3,021.46	1,859.18	1,497.82	1,851.19	382.85	338.28	326.95

The alpha diversity analysis indicated that species richness was generally greater in rumen samples, though this difference was not significant in the winter 2023 samples (Figure 1A). The variations in significance levels were attributed to differences in grazing times during different seasons and subsequently the rumination period, which influenced the capture of rumen microbiota in the oral cavity, despite sample collection taking place within similar timeframes. Beta diversity, visualised through principal component analysis (PCoA), revealed distinct clustering between oral and rumen samples across different seasons (Figure 1B).

Permutational multivariate analyses of variance (PERMANOVA) were conducted to evaluate how season, sample type, and individual animal contribute to the beta diversity differences between oral and rumen samples (Table 2). These analyses confirmed significant differences ($P < 0.05$) in overall microbiome composition between oral and rumen samples at multiple taxonomic levels, including Phylum, Class, Order, Family, Genus and Species (result not shown). The differences may be attributed to the different physiological functions of the oral cavity and rumen. Additionally, the rumen is a specialised, nutrient-dense anaerobic environment that fosters a diverse yet distinct community of symbiotic microorganisms essential for the rumination process. The oral cavity, by contrast, is a more dynamic environment due to its anatomical position. Constant fluctuations in pH, nutrient availability, and moisture levels restrict the establishment of a diverse microbial community. However, when the PERMANOVA analysis focused specifically on the phylum *Euryarchaeota*, which contains mainly the methanogens, the compositional differences between oral and rumen samples were not significant ($P = 0.19$) (Table 2). Visualising the relative abundances of the top 5 genera within *Euryarchaeota* further highlighted the similarities in methanogen composition between oral and rumen microbiomes (Figure 2). These findings suggested that, despite the overall differences in microbial communities, oral samples captured the methanogen profiles during rumination. This study showed that the methanogen profiles were consistent between oral

and rumen samples, supporting the potential for using oral samples as a practical proxy for rumen sampling in methane-related studies.

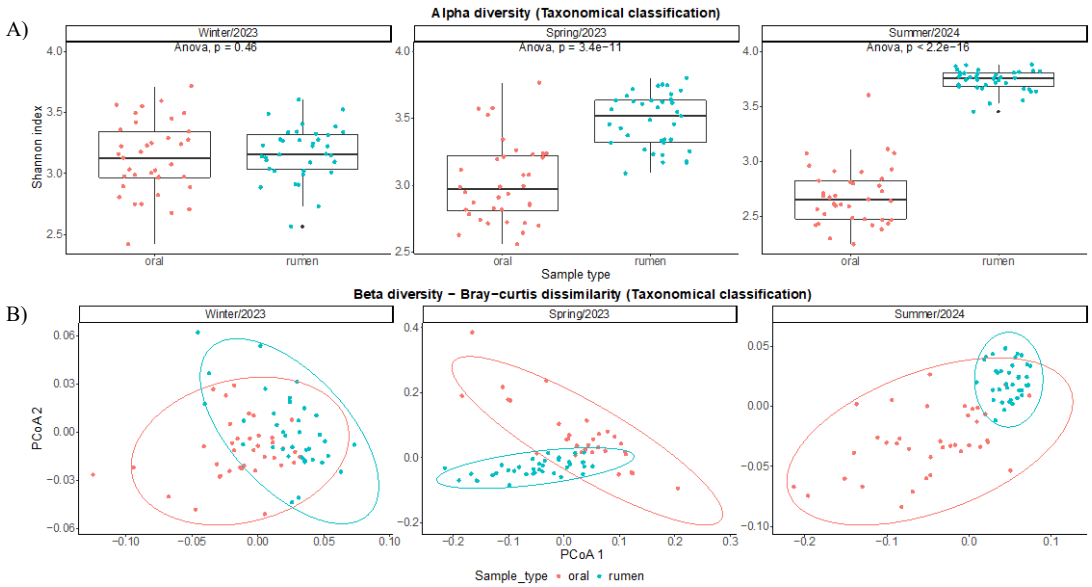

Figure 1. A) Alpha diversity and (B) beta diversity of the oral (red) and rumen (blue) microbiomes collected from 18 empty cows across 3 different timepoints, winter/2023, spring/2023 and summer/2024 in Queensland Australia

Table 2. F statistic, coefficient of determination (R^2) and *P*-values for season, sample type and animal from PERMANOVA analysis testing the differences in beta diversity between oral and rumen samples

Subset	Variable	R^2	F statistic	P-value	Significance
Whole	Season	0.40388	79.3041	0.000999	***
	Sample type	0.0823	32.4109	0.000999	***
	Animal	0.05779	0.5973	0.995005	
Phylum:Euryarchaeota	Season	0.02692	2.8846	0.01099	*
	Sample type	0.00751	1.6085	0.19181	
	Animal	0.13027	0.7346	0.96803	

This study examined the methanogen profile to demonstrate the potential of using the oral microbiome for methane emission research. However, the relative abundances of the top 5 genera in the *Euryarchaeota* subset were low, likely due to the limited sequencing coverage in this study. To address this limitation, future studies should sequence microbiome samples at greater depth to enhance coverage. Additionally, methanogenesis may be taxonomically more diverse than previously assumed (Wu *et al.* 2024). Recent research indicates an advancement in the functional meta-omics and the benefit of its integration in the methane emission studies (Zhao *et al.* 2024). Therefore, future investigations should incorporate accurate methane emission measurements and explore both taxonomic and functional subsets of the metagenome to identify key microbiome components significantly associated with the methane emission phenotype.

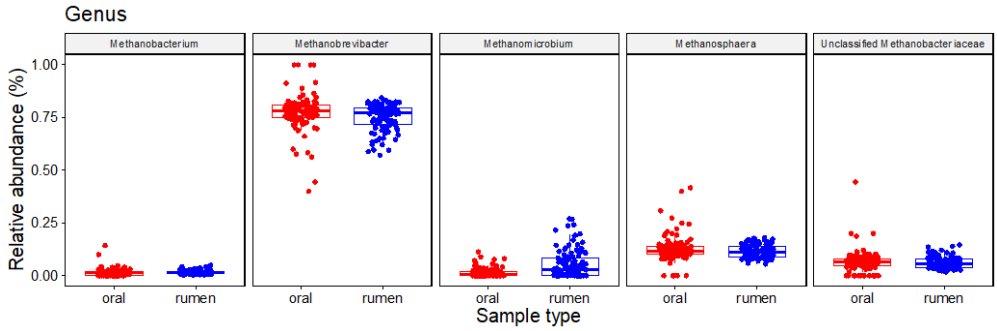

Figure 2. The relative abundances of the top 5 genera in Euryarcheota subset of the microbiome samples collected from oral (red) and rumen (blue) samples collected from 18 empty cows across 3 consecutive seasons, winter/2023, spring/2023 and summer/2024

Previous studies suggested the high similarity between oral and rumen samples when sampling was conducted at a certain timepoint under controlled conditions (Young *et al.* 2020). In this study, significant differences in the diversity and composition of microbiomes from oral and rumen samples collected from grazing animals across different seasons highlight the challenges of using oral samples as a proxy for rumen microbiomes in grazing animals. However, the study proposes that a strategic data analysis approach, such as focusing on methanogen profiles, could overcome these limitations for studying methane emissions. This approach may also be applied to other studies aiming to replace rumen sampling with oral samples, though establishing the most effective analysis strategy requires extensive background research.

CONCLUSION

While oral samples have been proposed as a proxy for rumen microbiomes, this study highlights the challenges of applying this approach to grazing animals. Significant differences in diversity and composition between oral and rumen microbiomes were observed in this study. However, the notable similarity in methanogen profiles suggests that, with strategic data analysis and further research, oral microbiome sampling holds potential as a practical alternative for rumen microbiome studies in methane emission research.

ACKNOWLEDGEMENTS

This work was made possible by the dedication of the LESTR group, UQ QASP staff, and funding from Meat and Livestock Australia (project P.PSH.2010)

REFERENCES

Kumar A., Skrahina V., Atta J., Boettcher V., Hanig N., Rolfs A., Oprea G. and Ameziane N (2023) *Front. Genet.* **14**: 1081424

Ross E.M., Nguyen L.T., Lamb H.J., Moore S.S. and Hayes B.J. (2022) *bioRxiv*, **2022.02.09**.479458.

Wu K., Zhou L., Tahon G., Liu L., Li J., Zhang J., Zheng F., Deng C., Han W., Bai L., Fu L., Dong X., Zhang C., Ettema T.J.G., Sousa D.Z. and Cheng L. (2024) *Nature* **632**: 1124.

Young J., Skarlupka J.H., Cox M.S., Resende R.T., Fischer A., Kalscheur K.F., McClure J.C., Cole J.B., Suen G. and Bickhart D.M. (2020) *Appl. Environ. Microb.* **86**: e00861.

Zhao Y., Tan J., Fang L. and Jiang L. (2024) *Sci. Total Environ.* **951**: 175732.