BRIEF COMMUNICATION: Exploration of the New Zealand Deer Rumen Microbiome

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Abstract
Rumen microbial communities (RMC) have been shown to be affected by the host individual's genetics and are heritable in sheep and cattle. RMC are predictive of feed related traits such as methane emissions, a by-product of the fermentation process. The aim of this project was to determine the variability in RMC amongst deer and to estimate heritability of the deer RMC. The rumen contents of 409 red and wapiti cross deer were sampled, and microbial DNA was sequenced. The genome taxonomy database was used to determine taxonomy assignment at the genus level. Sequences were used to determine similarity of RMC amongst individuals and presence of known microbes. Similar to other ruminants, *Prevotella* was the most abundant genus present in the deer RMC. Dimension reduction methods were applied to three standardisation methods to examine the variation in the RMC. Heritability estimates for the first two components of the reduced dimensions of the RMC ranged from 0.078 (± 0.058) to 0.72 (± 0.21) across the standardisation methods. This suggests that the deer RMC is controlled by host genomics.

Keywords; Deer, Rumen, Microbiome, Heritability

Introduction

Grazing ruminants can digest complex carbohydrates such as plant cell walls through a symbiotic relationship with microbes in the rumen. The fermentation pathways, along with the resulting energy sources and waste products, vary depending on the rumen microbial communities (RMC) present in the gut (Keum et al., 2024). A prevalent byproduct of this fermentation is methane, which can be predicted based on diet composition and the bacteria that drive fermentation pathways, producing varying amounts of hydrogen substrate (Clemmons et al., 2019, Rabee et al., 2024). Mitigation of methane emissions from ruminants can be achieved through selective breeding, where a key factor is the prediction of RMC (Hess et al., 2022). Although diet significantly influences RMC, they have been shown to be heritable in ruminants like sheep and cattle, with heritability estimates ranging from 0.1 to 0.4 (González-Recio et al., 2023). Therefore, RMC can be considered a phenotype for inclusion in selection. This has been done successfully in sheep, with the combination of host genetics and metagenomic profiles (Hess et al., 2022). Whilst the deer RMC have not been as intensively explored as other ruminants, it has been associated with disease immunity, resistance to pathogenic bacteria, and feed efficiency (Hu et al., 2017). Hence, the deer RMC may also be heritable and be used to increase prediction accuracy of breeding values in deer.

Deer are highly adaptable and versatile animals, thriving in a broad range of environments (Berlioz et al., 2017). The RMC of sheep and cattle have been shown to have similar heritability and be predictive of methane. Deer are browsers and adapt to seasons, therefore have different digestive characteristics than other ruminants. Hence, further investigations into deer RMC are crucial to expanding the industry and reducing methane emissions. The aim of this paper is to investigate variations in New Zealand deer RMC and estimate the heritability of the RMC.

Materials and Methods

Experimental animals and protocols applied in this study were approved by the AgResearch Ruakura (Hamilton, NZ) Animal Ethics committee (approvals 14906, 14907 and 14981).

Animals & phenotypes.

The rumen contents of 409 red and wapiti cross deer (*Cervus elaphus* and *Cervus canadensis* respectively) from three different cohorts across New Zealand were sampled over three years. SNP data was generated for individuals using genotyping by sequencing (GBS) and sex, breed and year of birth (YOB) were also recorded.

Rumen microbial counts.

Microbial DNA was sequenced using restriction enzyme reduced representation sequencing (Hess et al., 2020). Sequences were processed through the reference-based pipeline described in Hess et al. (2020) using the genome taxonomy database (GTDB) (Parks et al., 2021) to classify microbial sequences at the genera level. The microbial count matrix was created using relative abundance of genera per sequence, and filtered so that only genera that had a relative abundance greater than 0.01% within at least one cohort were retained.

Statistical models.

Three standardisation methods were applied to the microbial count matrix. (1) Microbial counts were converted to a relative abundance matrix (i.e., counts were converted into proportions per sample), log10 transformed and scaled per genera within each cohort, then converted into a microbial relationship matrix (MRM) as described in Hess et al. (2020), (2) A centred-log ratio (CLR) transformation was applied to the microbial count matrix (creates a covariance matrix reducing the distance between counts to a normalised scale). (3) A Bray-Curtis dis-similarity matrix was generated using the CLR transformed matrix from (2) (measures dis-similarity between samples on a scale from 0-1). Different standardisation methods were used to ensure that heritability of the RMC was not an artifact of a particular standardisation method. Cohort correction ensured population stratification was removed. Principal Component Analyses (PCA) were applied to the MRM and CLR adjusted count matrices and Principal Coordinate Analyses (PCoA) were performed using the Bray-Curtis matrix as a distance matrix. The heritability of the first two components for each method were estimated using a linear mixed model with YOB, sex and breed fitted as fixed effects, and additive genetic effect using a genomic relationship matrix as variance/covariance matrix, where the response variable is the first or second component from the PCA or PCoA.

Results

The most prevalent genus in the deer RMC was *Prevotella* and the top ten most abundant genera make up ~50% of the total microbial counts across all individuals (Figure 1). Cohorts from 2022 and 2019 are the most similar, with the top three most abundant genera (*Prevotella*, *Limivicinus*, and *Cryptobacteroides*) making up ~25% of the total relative abundance. The 2021 cohort displays higher levels of *Prevotella* overall with a relative abundance ranging from ~19%-30% of the total microbial counts across individuals. There is microbial count variation across individuals, where individuals within the same cohort are the most similar.

The first component for the Hess standardisation (method 1) explains 28% of variation amongst the MRM, whilst component two explains 14%. Component one for the CLR standardisation (method 2) explained 18% of the variation across the RMC, whilst component two explains 12%. The heritability's estimated from the components ranged from 0.078-0.72 (Table 1). Component 1 displayed a higher heritability estimate than component two for methods 2 and 3. Component two was found to have a higher heritability estimate than component one in method 1. The standard errors for both components for method 3 were significantly lower than those found in methods 1 and 2. Component one of method 2 had the highest heritability estimate of 0.72 ± 0.21. Component two of method 3 had the lowest heritability estimate of 0.078 ± 0.058.

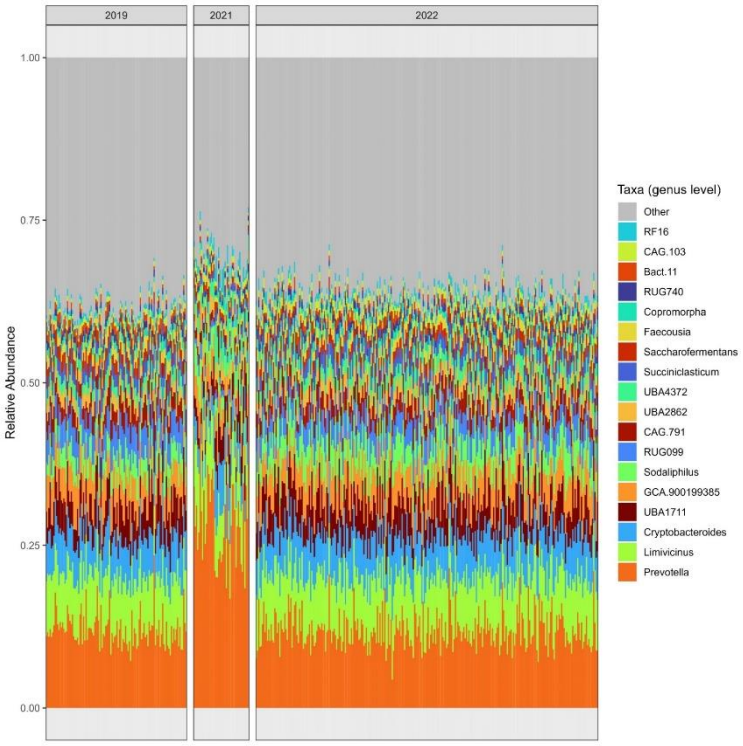

Figure 1 Relative abundance plot of rumen microbes by year of collection. Samples are placed across the x axis and relative abundance of genera on the y axis. The most abundant microbes are on the bottom, with decreasing abundances further up, where the total composition of genera adds to 1. Microbial genera are separated by colour and given on the right, most abundant on the bottom and moving upwards with least abundant.

Table 1. RMC heritability estimates per standardisation method. The response variable gives the components explaining the largest amount of variance in the model (1 and 2), with the proportion of variance explained in brackets.

Standardisation method	Response variable (component)	h^2	SE
Hess (1)[1]	1 (0.28)	0.17	0.14
	2 (0.14)	0.57	0.15
CLR (2)[2]	1 (0.18)	0.72	0.21
	2 (0.12)	0.44	0.25
Bray-Curtis (3)[3]	1 (-)	0.65	0.047
	2 (-)	0.078	0.058

[1]The Hess method (1) log corrects per cohort then scales per genera.

[2]The CLR method (2) creates a correlation matrix with CLR standardisation.

[3]The Bray-Curtis method (3) creates a dis-similarity matrix from the matrix in method 2.

Discussion

The most prevalent genera in the deer RMC was *Prevotella*, which is consistent with findings in cattle, goats, sheep and in farmed red and fallow deer (González-Recio et al., 2023, Wang et al., 2019, Rabee et al., 2024). In cattle *Prevotella* was observed to make up to 60% of the RMC, providing evidence of persistence across a variety of diets (Keum et al., 2024). *Prevotella* has been found to improve volatile fatty acid production which is a preferred energy source for ruminants (Rabee et al., 2024). *Prevotella* has been associated with higher methane production, however, due to being the dominant species, this does not inform much about manipulating methane output (Han et al., 2023). Another highly abundant genus was *Sodaliphilus*, which is associated with methane production in dairy calves, although the mechanism for this is largely unclear due to the ambiguity of its role in the rumen microbiome (Park et al., 2022). Therefore, the deer rumen microbiome shows similarities to that of other ruminants and may be used to predict methane emissions. The RMC was seen to vary across individuals, but the largest variation observed between cohorts. This is likely due to the individuals within cohort having the same environment and being in close contact (Wang et al., 2019). Because of this, the cohort effect has been corrected for heritability estimation.

The standardisation methods depict different aspects of the rumen microbial composition data due to the transformations undertaken. The Hess method (method 1) measured the relationships in the RMC between individuals adjusting for cohort variation. The CLR method with PCA (method 2) describes the most variable aspects of the RMC. The Bray-Curtis method (method 3) measures the dissimilarity in the RMC between individuals. The heritability estimates for the components for each model varied significantly ($0.078 \pm 0.058 - 0.72 \pm 0.21$). This is likely due to the first two components from the PCA or PCoA capturing different aspects of the RMC. However, since each method has a component with a significant non-zero heritability estimate, we conclude that aspects of the deer RMC is controlled by the host genome.

This study has successfully shown that components of the deer RMC are heritable. This knowledge may be used to improve breeding selection. Further research is required to investigate whether the RMC is associated with key production traits.

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