BRIEF COMMUNICATION: Genetic control of the rumen microbiome in sheep

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Introduction

Methane is a waste product generated by methanogenic archaea in the rumen of the host. In New Zealand, methane emitted by ruminants accounts for ~31% of anthropogenic greenhouse gas (GHG) emissions. Mitigation strategies to lower ruminant methane production are an important aspect of the national strategy to reduce GHG emissions. It has been shown that methane varies both with the genetics of the individual ruminant host and with the microbial populations present in the rumen. Current research is focussed on diet, vaccination, chemical inhibition, and genetic selection of lower emitting animals.

The mitigation of methane production by genetic selection has been shown to be effective by divergently selecting for high and low methane emitting sheep (Pinares-Patiño et al. 2011; 2013). This approach is attractive as breeding is cumulative and sustainable. The many thousands of methane measures required for a commercial breeding program, however, would be prohibitively difficult and expensive to collect. Methane measures in sheep require multiple days in specialist respiration chambers and currently cost ~$1000 per individual. For an effective breeding program to reduce methane on a national/international scale, alternative predictors are needed.

Although it is known that methane production varies with rumen microbial community structure (RMC) (Kittelmann et al. 2014; Ross et al. 2013;Wallace et al. 2014), it is not known whether the host genetics also control the structure of RMC or whether RMC could be used to predict methane directly. The aim of this research was to evaluate rumen microbial community profiles sampled from the rumen as a tool to predict gross enteric methane emissions (g CH_4 /day) and methane yield (g CH_4/kg dry matter intake (DMI)).

Materials and methods

A full description of the animals used together with the materials and methods for measuring methane emissions can be found in Pinares-Patiño et al. (2011). Two rumen contents samples were collected by stomach tube from each of 259 New Zealand crossbred sheep at ~10 months of age. Rumen samples were collected 14 days apart approximately 18 hours after the last feed. The sheep were from selection lines phenotypically divergent for methane emissions per unit of dry matter intake (CH_4/kg DMI) and were measured in closed-circuit respiration chambers for methane emissions continuously for 48 hours prior to each rumen sampling (Pinares-Patino et al. 2013). Sheep were offered a ration of lucerne pellets based on 2.0 × maintenance energy requirements. Feed was offered twice daily and individual dry-matter intakes were recorded.

To determine the RMC of individual rumen samples, microbial DNA was extracted, and sequencing of bacterial 16S rRNA genes was performed on a 454 Genome Sequencer. This has been described previously by Kittelmann et al. (2013). Sequence data were phylogenetically assigned by using the BLAST algorithm to compare against a reference sequence database (McDonald et al. 2012). After exclusion of taxa that were detected at relative abundances of <1% in all samples, the vast majority of sequences were assigned to 54 taxa. Each individual profile, therefore, consisted of the relative abundance or proportion of each of the 54 taxa.

To reduce the dimensionality of the data set, and to facilitate comparing RMC profiles between individual sheep, correspondence analysis (CA) was used (Greenacre 1984). The co-ordinates (say, CA1 & CA2) of the first and second CA dimensions (ranked by variance or inertia explained) were used as intermediate phenotypes to represent each profile as by Kittelmann et al. (2014). These metrics were used to compare RMC amongst individuals, and to determine the heritability and repeatability of microbial community structure. Genetic parameters, phenotypic and genetic correlations between RMC and methane emissions were determined with univariate and bivariate mixed linear models. Sex, birth rearing rank, age and cohort (based on rearing and assignment to measuring groups) were accounted for within the model. Pedigree-based relationships were used to fit a polygenic animal effect.

In an alternative approach, one using the full dimensionality of the data set, Hellinger distances (Hazewinkel 2001) between microbial profiles were calculated. These were used to summarise the differences between profiles across both animals and time.

Results and discussion

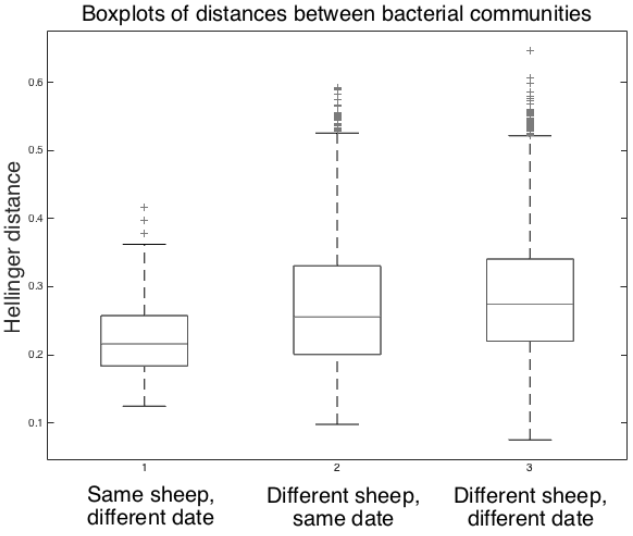
Mean gross methane emissions (g CH_4/day) and methane yield (g CH_4/kg DMI) were 24.10 (±0.10) and 15.06 (±0.05) with coefficient of variation of 0.14 and 0.11 respectively. Preliminary results showed evidence

Table 1 Heritabilities, repeatabilities, genetic and phenotypic correlations for rumen microbial communities (defined by first and second components of correspondence analysis CA1 and CA2) and methane emissions measured as CH4d (g methane per day), and CH4DMId (g methane per kg dry matter intake measured over a day) in sheep.

Traits (trait 1 trait 2)	h^2 trait 1	s.e.	h^2 trait 2	s.e.	rep trait 1	s.e.	rep trait 2	s.e.	r_g	s.e.	r_p	s.e.
Univariate single trait analyses												
CH4d	0.38	0.18			0.76	0.03						
CH4DMId	0.42	0.13			0.51	0.05						
CA1	0.24	0.12			0.51	0.05						
CA2	0.13	0.09			0.45	0.05						
Bivariate – 2 trait analyses												
CH4d CH4DMId	0.4	0.18	0.4	0.13	0.76	0.03	0.51	0.05	0.47	0.22	0.64	0.04
CA1 CH4d	0.22	0.11	0.27	0.16	0.51	0.05	0.75	0.03	0.58	0.42	-0.14	0.06
CA2 CH4d	0.13	0.09	0.27	0.16	0.45	0.05	0.75	0.03	0.77	0.44	-0.04	0.06
CA1 CH4DMId	0.24	0.12	0.41	0.13	0.51	0.05	0.5	0.05	0.06	0.32	-0.3	0.05
CA2 CH4DMId	0.1	0.08	0.4	0.13	0.45	0.05	0.5	0.05	0.9	0.35	0.06	0.05

Where h^2 is the heritability (additive genetic proportion of phenotypic variation), rep = repeatability which is the sum of the heritability and the permanent environment, r_g is the additive genetic variance and r_p is the phenotypic correlation of trait 1 with trait 2. Correlation is based on $cov_{12}/sqrt(var_1 var_2)$.

Figure 1 Boxplots of the dissimilarity of bacterial communities for three (progressively more distant) classes of sheep pairs. The left-hand boxplot shows the dissimilarities within sheep after one day, the central boxplot shows the dissimilarities between sheep on the same day and the right-hand boxplot shows the dissimilarities between different sheep after one day. The difference in bacterial communities within the same sheep over one day is generally less than that measured between different sheep at the same time, which in turn is generally less than that between different sheep at different times.



for heritable variation of RMC and for genetic covariance of methane emissions and RMC. Table 1 gives moderate heritabilities of ~0.24 ± 0.10 and 0.13 ± 0.09 for CA1 and CA2, respectively. Repeatabilities (based on two measures per animal) were 0.51 ± 0.05 for CA1 and 0.45 ± 0.05 for CA2. Thus, the RMC trait appears to be heritable and repeatable. Furthermore, the distance analyses showed that RMC profiles in samples obtained from the same sheep at different times were more similar than those obtained from different sheep on the same date or on different dates (Figure 1).

The extent to which animals differed in methane emission was correlated with differences in microbial profiles. Genetic correlations were much higher than phenotypic correlations, indicating that the traits are subject to considerable environmental noise. The genetic correlation was greatest with CA2 for both gross methane emissions (0.77±0.44) and for methane yield (0.9±0.35) indicating that ~80% of the variation in methane yield could be explained by differences in RMC. Standard errors are high and these results require validation in larger data sets, however, these findings are consistent with previous analyses (Kittelmann et al. 2014), suggesting that the first and second principal coordinate divided low methane emitting animals into two groups with differing microbial structures. Our results suggest that microbial community structure may be influenced by host genetics and could be a useful predictor of methane production in sheep. Further, rumen microbial communities may also be potential predictors for many other traits related to rumen stoichiometry and feed intake. Further work is required to test whether the genetic parameters and correlations hold for different diets and crucially for animals fed on pasture.

Future work will involve the generation of a larger data set to enable more complex statistical analyses of the data and the modelling of high-density host-marker genotypes to look for specific regions of the host genome that control rumen microbial communities.

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