Rumen Differences between Sheep Identified as being Low or High Methane Emitters

W. E. Bain[1]*, L. Bezuidenhout[1], N. B. Jopson[2], C. Pinares-Patino[3], J. C. McEwan[1].
[1]AgResearch Invermay, Private Bag 50034, Mosgiel, 9053 New Zealand, [2]AbacusBio, PO Box 5585, Dunedin, 9058, New Zealand, [3]AgResearch Grasslands, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand

ABSTRACT: Divergent selection lines of sheep for methane emissions have been established. The top and bottom 10% of ewes, (gCH_4/kgDMI) screened for this trait, were computer tomography scanned (24 high and 21 low emitters). The following rumen compartments; reticulum, rumen and atrium, ventral sac of rumen (rostral), dorsal sac of rumen and ventral sac of rumen (caudal) were measured for volume, and surface area. Rumen composition (gas, raft, liquid) volume and weight were also measured. Data were analysed using R, and parsimonious models selected for different variables. Measurements between the two groups differed with high methane emitters having greater total rumen surface area (12%), total rumen volume (20%), and total rumen raft volume and weight of rumen contents (27%, 28% respectively). Results suggest that differences in CH_4 yield between low and high CH_4 emission sheep arise from morphological differences of their reticulo-rumen.

Keywords:
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Introduction

Methane (CH_4) is known to be one of the main greenhouse gases contributing to climate change. Livestock enteric emissions of CH_4 are a major source contributing 35% of greenhouse gas (GHG) emissions in New Zealand, mostly from ruminal fermentation (Pinares-Patiño et al. (2009)). Many approaches have been investigated to help mitigate livestock CH_4 emissions for pastoral farming systems (Oddy et al. (2014)). Breeding animals for lower CH_4 yield emissions per unit feed dry matter intake (g/kg DMI) may be a practical strategy for reducing CH_4 emissions. Research has shown that gross emissions (g CH_4/day) and methane yield are heritable and repeatable (Pinares-Patiño et al. (2013)). Most of the enteric CH_4 is produced in the rumen and studies have indicated that low CH_4 yielding sheep have smaller rumens, lesser rumen contents and shorter mean retention time than high CH_4 yielding counterparts (Barett et al. (2012); Pinares Patiño et al. (2003) Goopy et al. (2013);). This suggests that although breeding animals for lower CH_4 yield may be a suitable strategy for reducing CH_4 emissions, subsequent effects on rumen morphology and function should be determined.

Respirations chambers (Pinares-Patiño et al. (2008)), which facilitate accurate measurement of CH_4 emissions, and were used to screen animals to establish selection lines for high and low CH_4 emission (Pinares-Patiño et al. (2013)). Animals from the initial screening of these lines were used in this study to determine whether there were differences in rumen shape and volume between high and low CH_4 emitting sheep. The following parameters: rumen shape, size and composition were estimated using computer tomography (CT).

Materials and Methods

Animals. All animal procedures were carried out at AgResearch Invermay (Dunedin New Zealand) and were approved by the AgResearch Animal Ethics Committee. The selection lines were formed by screening CH_4 yield of animals (born 2007, 2009-2011) from central progeny test programme flocks (McLean et al (2006)). Breeding values were estimated using best linear unbiased prediction (BLUP) (Pinares-Patiño et al. (2013)). The 45 ewes (24 high and 21 low CH_4 emitters) in this study were selected from approximately 250 ewe lambs born in 2009; with methane measurements recorded using respiration chambers at 8-12 months of age. The top and bottom 10% of animals were assigned to a high or low selection group, respectively, based on their CH_4 yield. The differences between the born 2009 extremes at screening for CH_4 yield (g CH4/kg DMI) in 2010 using the method outlined by Pinares et al (2013) was 28% (high/low animals). Repeat measurements on the same animals in each of the 2 subsequent years were 10% indicating that these differences are repeatable over time. The flock is maintained at AgResearch Woodlands Research station (Southland New Zealand) and the animals were transported to AgResearch Invermay Agricultural Centre for CT measurements in June 2012.

Measurements. Live weight was recorded prior to transport. The animals were grazed together on ad lib pasture prior to measurement and removed from pasture at 9am on the morning of measurements. The difference between the time that an animal was removed from pasture and the exact time of scanning was recorded for each individual. Scanning took place over four days, hence day of scanning was also recorded.

CT Measurements. In preparation for scanning, sheep were sedated with Xlyase20 given by intramuscular injection (0.25-0.5mg/25kg body weight). The animals are scanned in a prone position using a custom built cradle (made of a 400mm diameter PVC pipe which has been halved with three webbing straps for restraint) with hind legs extended caudally and forelegs under the chest. The rumen was scanned using X-ray computed tomography (CT; Siemens Somatom AR.C, Siemens Medical System, Erlangen, Germany) using the procedures described by (Jopson et al. (1997)), with a random starting position

Table 1: Mean ± standard error of the mean of rumen total and rumen compartments measured from low CH4 (n=21) and high CH4 (n=24) emission animals. Units: Volume L, Weight Kg, Surface Area mm².

Region		High	Low	P value	% difference*
Total	**Volume**	**8.4 ± 0.3**	**6.7 ± 0.3**	**0.001**	**20**
	Surface Area	**2950 ± 69**	**2585 ± 74**	**0.001**	**12**
	Gas Volume	0.75 ± 0.07	0.80 ± 0.08	0.682	-7
	Raft Volume	**6.6 ± 0.3**	**4.8 ± 0.4**	**0.001**	**27**
	Raft Weight	**4.3 ± 0.2**	**3.1 ± 0.2**	**0.001**	**28**
	Liquid Volume	1.1 ± 0.1	1.1 ± 0.1	0.823	0
	Liquid Weight	1.0 ± 0.1	1.1 ± 0.1	0.834	-10
Reticulum	**Volume**	**1.3 ± 0.08**	**1.1 ± 0.08**	**0.048**	**15**
	Surface Area	495 ± 21	435 ± 24	0.068	12
Rumen and atrium	**Volume**	**4.6 ± 0.2**	**3.3 ± 0.2**	**0.001**	**28**
	Surface Area	**1356 ± 48**	**1121 ± 52**	**0.002**	**17**
Ventral sac of rumen (rostral)	Volume	0.57 ± 0.07	0.51 ± 0.07	0.509	11
	Surface Area	283 ± 19	262 ± 20	0.458	7
Dorsal sac of rumen	Volume	0.90 ± 0.08	0.95 ± 0.09	0.692	-6
	Surface Area	375 ± 24	374 ± 26	0.973	0.3
Ventral sac of rumen (caudal)	**Volume**	**1.03 ± 0.08**	**0.75 ± 0.09**	**0.020**	**27**
	Surface Area	440 ± 27	374 ± 29	0.094	15

*Percentage difference is the difference between the high and low as a proportion of the high.

approximately prior to the diaphragm at the 6th-7th thoracic vertebrae, through to the end of the rumen at around the 6th lumbar vertebrae. Images were collected at 15mm intervals, with a 450mm field of view and 3mm slice thickness. A total of 30-32 images were collected for each animal.

Images were processed individually to remove all other organs and divided into five compartments for further image processing. The compartments were the reticulum, combined atrium and main rumen compartment, rostral-ventral sac, dorsal sac and caudal-ventral sac.

The volume and surface area of each of the rumen compartments were estimated from the area and perimeter in each image using ImageJ (US National Institutes of Health, Bethesda, Maryland, USA), multiplied by the distance between images. Volume and weight were also estimated for the rumen fill components, namely gas, raft and liquid phases using AutoCAT (Jopson et al, (1995)). Image histograms were separated into these compartments using Hounsfield unit (HU) ranges of -1024 to -630, -629 to -114 and -113 to 228 HU for gas, raft and liquid, respectively. Area (count of pixels) and average pixel density were measured in each of the three groups. Volume was calculated for each of the three components by summation of the areas multiplied by the distance between images. Average pixel density was determined by weighting the average density in the individual images by the pixel area in each image. Pixel density was converted to physical density using the relationship shown between HU value and density by Fullerton (1980).

Statistical analyses. Data were analysed using R, (Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.), and parsimonious models selected for the different response variables. Models were compared based on the squared correlation coefficient (R^2), average information criterion (AIC) and residual standard deviation (RSD) values. Non-significant interactions were removed from the final models. Pairwise comparisons were run using Least Squares Means. The models fitted included fixed effects of treatment (high or low), day, and treatment*day interaction. Live weight and time difference were fitted as covariates. The interaction effect treatment*day was only significant for total gas volume and dorsal sac volume, whilst the interaction time difference was only significant for the volume and surface area of the reticulum.

Results and Discussion

Table 1 reports the measured parameters for the CH_4 selection lines. These differed in rumen total volume and surface area, total raft volume and weight. Differences in volume were also recorded for the following individual rumen compartments: reticulum, rumen and atrium, and caudal ventral sac of the rumen. High CH_4 emitters had 20% greater ($P<0.001$) total rumen volume and 12% larger ($P<0.001$) surface area than low CH_4 emitting animals. This trend is also evident in three of the five individual compartments measured, with the high methane emitters having greater volume in the reticulum, rumen and atrium, and caudal ventral sac of the rumen, of 15%, 28% and 27% respectively. This is consistent with (Goopy et al. (2013)) who reported that low methane emitting sheep had smaller rumens and lesser rumen contents (particulate plus liquid;). then there high emitting counterparts.

Composition of the total rumen was significantly different for raft total volume and weight between the high and low methane emitters ((P=0.001), with the high methane emitters having greater raft volume (27%), and weight (28%) respectively. There was no significant difference for the other two components, gas and liquid volume or liquid weight between the high and low methane emitters. Goopy et al. (2013), concluded that the association between rumen content morphology from differential methane emissions from fasted animals were inconclusive, these findings were based on visual scoring. The significant differences observed in this study were from quantifiable CT methodology and non-fasted selection line animals.

Conclusion

These preliminary findings indicate that rumen content, volume and surface areas differ significantly between high and low methane emitting sheep. This is the first study to investigate the rumen content/complexity by means of CT imaging, showing a significant difference between the raft phases of methane selection lines.

This study suggests that selection for reduced methane emissions has resulted in a correlated change in rumen size. Of considerable interest in any research is the repeatability of measurements hence further investigation is needed to confirm that the results are consistent across years, this study has been repeated and is in the process of being analysed

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