final report

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Breeding low methane sheep and eludicidating the underlying biology

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Abstract:

Genetic improvement is one method to reduce enteric methane production by grazing sheep. It is suited to extensive grazing systems where other forms of intervention are impractical. Measurements are required on large numbers of individual animals to estimate the impact of genes on methane production and establish correlations with other commercially important traits. We developed a short-term method for measuring methane in the field using portable accumulation chambers and screened over 3000 animals to estimate genetic parameters and identify high and low methane-emitting individuals to investigate their rumen physiology, rumen microbial ecology and net feed intake. Methane yield (production/kg DMI) is related to the time feed particles spend in the rumen and sheep with high methane yield have larger rumens than sheep with lower emissions. Our preliminary work has not identified any clear differences in the microbial ecology between high and low emitting animals. Sire variation was noted in a number of traits linked to feed but there is little evidence that feed efficiency explained variation in daily methane production. Our current estimates of heritability of methane output are low but there is enough evidence to suggest there is scope for industry to benefit through genetic selection and developing a methane index.

Executive summary:

Genetic improvement is one method to reduce enteric methane production by grazing sheep and one that is suited to extensive grazing systems where other forms of intervention are impractical. The aim of this research project (BCCH.1015) was to:-

1. To identify useful predictors of daily methane production (DMP) that can be applied to large populations of individual sheep to enable the generation of genetic parameters for a methane trait.
2. To apply that method to genetic resource flocks available in Australia to establish preliminary estimates of phenotypic and genetic correlations between methane emissions and production traits. Flocks used were sire reference flocks Kattaning WA, those used in the SheepGenomics study and the Information Nucleus Flock –INF - managed by the Sheep CRC.
3. To identify high and low methane-emitting individuals using in-field screening and chambers and use them to compare their rumen physiology, rumen microbial ecology and net feed intake (NFI) to improve our understanding of the underlying biological basis for differences in methane production.

The general experimental design was to develop a method that predicts daily methane production, use it to screen large number of individual animals under 'field' conditions to estimate genetic parameters, and identify 'high' and 'low' emitting animals that could be used to elucidate some of the underlying biology behind that the trait. More specifically, the 'high' and 'low' methane emitting animals were used for physiology and rumen kinetic studies and to collect rumen fluid samples for ruminal microbial analysis. These animals were also amongst a number of animals screened for net feed intake to establish whether there was any link between animals that were more feed efficient and methane production.

We found that a 1 hour measurement of methane production was a useful predictor of daily methane output from sheep when compared to any rumen fluid or breath-based predictors. The correlations between short-term and daily methane production were dependent on time of day (after feeding) and ranged from r= 0.50 – 0.82 (average r = 0.77 (restricted) and r= 0.77 – 0.92 (*ad. libitum*). If time of sample was allowed for, consistent prediction of DMP (r>0.6) using a 1 hr test was possible. We designed and safety tested portable accumulation chambers (PACs) for short-term methane measurements and validated them against respiration chambers. Approximately 44% of the variation (r=0.66) in DMP was accounted for by the 1h MP measure and the repeatability of the short-term method ranged from 0.3 over weeks up to 0.5 over consecutive days. We concluded that 1hr measures of CH_4 output in portable static chambers were potentially useful for determining genetic parameters affecting CH_4 production. Accordingly we established an initial field protocol based on standardised overnight fast & 1 h feeding pre-measurement, enabling up to 90 sheep (in batches of up to 15 at a time) to be measured during the day.

We used this protocol to screen 1356 animals from key national genetic resource flocks (Sheep Genomics Flock, Central Test Sire Evaluation (CTSE) flock, Sheep CRC Information Nucleus Flock) and obtained an initial estimate of heritability of 0.13. Repeatability was approximately 0.47 or 0.32 if methane production was adjusted for live-weight. The highest and lowest 150 animals (200 in NSW and 100 in WA) were selected from this screening to investigate the underlying physiology, rumen kinetics and microbial ecology.

In the initial data collected (above) there was substantial variation in the amount of feed eaten in the hour before measurement, which contributed to variation in methane output. A modified protocol in which sheep were not required to be fasted or fed before measurement. This protocol was more repeatable (0.56) than the initial protocol and was used to screen animals from the INF. Based on the correlation between 1 and 23 hour measurements ($r>0.6$) and an assumed heritability of 0.15, it was calculated that by screening 2000 animals would be sufficient to obtain preliminary estimates of genetic correlations with other production traits. Accordingly we measured 2600 animals at 5 INF sites – Cowra, Kirby, Rutherglen, Trangie and Katanning. There were substantial differences in variances between sites. There was a small amount of genetic (sire) variation in methane (L/hr) adjusted for live-weight. Estimates of heritability (of methane output) in the IN Flock ranged from 0.03 to 0.05. This was lower than we had hoped for based on our previous estimate and made estimating accurate genetic correlations impractical. Phenotypic correlations to production traits are currently being estimated.

Twenty (10 highest and 10 lowest methane-emitting after adjustment for LW) animals originally from the Sheep Genomics flock on which repeated 1 hr and 23 hr chamber measures had been made were selected to study the underlying biology of the methane trait. We measured aspects of physiology, rumen kinetics and rumen microbial ecology. Our results show that methane production is related to the time feed particles spend in the rumen (or the proportion of ingested feed retained in the rumen) and high methane emitting sheep have larger rumens than sheep with lower emissions. However, the proportion of rumen (organ) volume as gas and the estimated dry matter percentage of the rumen is similar between high and low emitters. This is consistent with the notion that rate of passage of feed through the rumen is one factor contributing to systematic variation in methane production.

In a separate study we have demonstrated that increased plasma concentration of the thyroid hormone T3 within physiological levels reduces retention time of rumen digesta and is associated with a reduction in enteric methane yield.

Rumen samples from animals divergent in methane output were analysed by microbial profiling (using both RFLP and sequencing). There were no clear differences between high and low emitting animals.

We also explored the potential for associations between feed use efficiency and methane production. Over 900 animals from different resource flocks have been measured for liveweight gain, feed intake, feed conversion ratio, residual feed intake and methane emissions using the PACs. Across all experiments, the average feed intake, liveweight gain, feed use efficiency and daily methane production of progeny from different sires varied by 30 to 40%. The difference in methane production were still evident after adjustment for differences in liveweight of progeny. There was little evidence that feed use efficiency, either RFI or FCE, explained significant variation in daily methane production. Daily methane production was most closely related to average liveweight ($r^2 = 0.26$; $P<0.001$) and liveweight change ($r^2 = 0.18$; $P<0.001$) during *ad libitum* feeding, and only weakly related to average feed intake ($r^2 = 0.04$; $P<0.05$). Further work is required to quantify the effects of feed intake over shorter periods immediately prior to methane measurements and undertake a combined analysis across all experiments to quantify phenotypic and genetic associations with both total methane production and methane production per unit of dry matter intake.

Although our current estimates of heritability of methane output are low and there is G x E variation that needs to be investigated there is scope for industry to benefit through genetic selection and a methane index. Our results suggest the PAC

protocols require further modification to include feed intake, and repeated tests over time to improve our prediction of daily methane production by individual animals and the likelihood of estimating genetic correlations to other commercially important production traits. However, the PACs are a novel development and the potential uses of this system are beginning to be explored both in Australia and overseas (NZ, UK). At present there is not enough confidence in estimates of genetic parameters for methane output to permit ASBVs to be reliably estimated. We have identified a number of issues that need to be further explored before we can confidently deliver information on genetic methods to reduce enteric methane emissions to industry.

1. The ewe flock contributes more than 70% of total enteric methane emissions from the Australian sheep industry. We don't yet know the best time to measure ewes to represent annual methane emissions and have planned a study to determine this in conjunction with feed intake and liveweight.
2. The short-term measurement (PAC) will be combined with measures of feed intake and possibly CO_2 output to improve a measure of the propensity of an individual to generate methane.
3. Further work on associations between methane emissions and physiology (e.g. rumen size, kinetics of digesta flow, microbial composition) should continue to identify likely mechanisms by which selection on a low methane phenotype may work. The advantage of continuing this work may provide an early warning sign in the event of negative associations between reduced methane emissions and potential productivity.

Background

Genetic improvement is one method to reduce enteric methane production by grazing sheep and one that is suited to extensive grazing systems where other forms of intervention are impractical. The aim of this research project (BCCH.1015) was to use sire referencing flocks and the information nucleus flock (INF) accessible to the CRC for Sheep industry Innovation to estimate the heritability of divergent methane emission from sheep and to establish preliminary estimates of phenotypic and genetic correlations between methane emissions and production traits. There have been 3 specific objectives of this project:

(1) To identify useful predictors of daily methane production that can be applied to large populations of individual sheep to enable the generation of genetic parameters for a methane trait.
(2) To apply that method to genetic resource flocks available in Australia to establish preliminary estimates of phenotypic and genetic correlations between methane emissions and production traits.
(3) To identify high and low methane-emitting individuals using in-field screening and chambers and use them to compare their rumen physiology, rumen microbial ecology and net feed intake (NFI) to improve our understanding of the underlying biological basis for differences in methane production.

The overall experimental design was to develop a method that predicts daily methane production, use it to screen large number of individual animals under 'field' conditions to estimate genetic parameters, and identify 'high' and 'low' emitting animals that could be used to elucidate some of the underlying biology behind that the trait. The 'high' and 'low' methane emitting animals were used for physiology and rumen kinetic studies and to collect rumen fluid samples for ruminal microbial analysis. Some of these animals were also amongst those animals screened for net feed intake.

This report is divided into 8 sections. The first 4 sections provide a summary of the research undertaken in this project: (1) the development and validation of the portable accumulation chambers and their use to screen genetic resource flocks and estimate genetic parameters, (2) the physiology and rumen kinetics of high and low emitting animals, (3) the microbial ecology of high and low emitting animals, and (4) linking net feed intake to methane production. Each section is linked to full text papers/reports as appendices for a complete description of the methods, results and full discussion. There are additional sections on the papers accepted for publication in peer review journals, media coverage and the integration we've had to other projects within the RELRP program.

Section 1. The development and validation of the portable accumulation chambers and their use to screen genetic resource flocks and estimate genetic parameters
(Link to Appendix 1 - 4)

To identify useful predictors of daily methane production, we used sheep in respiration chambers that were equipped with feeding devices to enable a feed delivery schedule that simulated grazing patterns of free-range sheep (4 x hourly feeds both AM and PM). We measured of a complete range of potential predictors of daily methane production (including rumen samples 10 times/d) in 12 sheep on each of 3 feeding levels (0.7, 1.05, 1.4 x Maintenance). The parameters we measured included VFA concentrations, rumen pH, CO_2:CH_4, 3 minute breath tests as well as partitioning the 22 hours the animals were in chambers into shorter 1-2 hour periods. Our results indicated that the short 1-2 hour measurement of methane production was the best predictor of daily methane output from sheep when compared to any rumen fluid or breath-based predictors that were considered (Appendix 1-2; Goopy et al. 2009, Robinson et al., 2010). The robustness of the1-2 hour measurement as a predictor of daily methane production was examined on different diets and feeding feeding regimes (*ad. libitum* vs restricted). The correlations between the short-term and daily methane production were dependent on time of day (after feeding) and ranged from 0.50 – 0.82 (restricted) and 0.77 – 0.92 (*ad. libitum*). There was always a strong association, so that if time of sample was allowed for, accurate prediction of DMP was considered possible.

Based on this we designed and safety tested portable accumulation chambers (PACs: 15 each in NSW and WA) for short-term methane measurements (Appendix 3; Goopy et al., 2011). Initial calibration of 15 sheep that had been measured for DMP then put in a field booth for one hour, showed a slightly lower correlation (r^2 = 0.50) than observed when a simple 1 or 2h portion of 24h respiration chamber was dissected (Figure 1). The validation was extended to 87 animals through two major studies. In Armidale, 40 sheep were fed a restricted diet and measured for three 22 h sessions (consecutive days) in respiration chambers and immediately placed in PACs for 1h. Regression analysis showed 43% of the variation in DMP was accounted for by the 1h MP measure. A similar study was conducted in WA with 47 Merino wethers but animals were fed *ad libitum* and fed twice in respiration chambers 4 weeks apart. In this case, the 1h MP accounted for 44% of the variation in DMP. Under these conditions the repeatability of the short-term method was approximately 0.5 in the short term (consecutive days) and 0.3 in the longer term (weeks – months), compared to the chambers which varied from 0.6 – 0.9 in the short term (consecutive days) and 0.3 - 0.5 in the long term (weeks – months). We concluded that 1 and 2 h measures of CH_4 output in portable static chambers were useful for determining genetic differences in CH_4 production in groups of ruminants and established an initial field protocol based on standardised overnight fast & 1 h feeding, enabling multiple groups of sheep to be measured in succession during the day.

The field-based protocol was used to screen 1356 animals from key national genetic resource flocks (n = 708, Sheep Genomics Flock, Falkiner station, Deniliquin, NSW; n = 448, Central Test Sire Evaluation (CTSE) flock, GSARI, Katanning in WA; n = 200, Sheep CRC Information Nucleus Flock in WA). We obtained an initial estimate heritability of approximately 0.15 (Robinson et al., 2010) and this was reasonably consistent between analyses in NSW and WA. Phenotypic correlations with feed intake, liveweight and rumen pH were estimated and it was clear that the main

influence on methane produced during the one-hour measurement was feed intake. Approximately 440 animals were re-screened at least once and in some cases three times to estimate repeatability of the measurement. Repeatability was slightly different between NSW and WA depending on whether or not data was corrected for feed intake, but was in the range of 0.47 or 0.32 if methane production was adjusted for liveweight. However, when 100 of the highest and lowest emitters were transported to Glen Innes and re-tested, animal effects remained, but not the previously-observed effects related to the animals' sires. Substantial variation was noted in the amount of feed eaten in the hour before measurement, leading to concerns that the test procedure might be increasing the variation in feed intake and hence methane emissions, making it unrepresentative of an animal's normal behaviour. Measurements were therefore made on the same animals using a modified test, in which sheep were measured directly off-pasture. By measuring over 160 sheep three times from pasture, this protocol was found to have a higher repeatability (0.56) than the previous 'fast and feed' protocol. It was on this basis, (i.e. that the 1h methane production explained approximately half of the variance in daily methane production and the repeatability of the test itself was approximately 0.56) that we chose to invest in applying the method to screen animals from the INF. It was estimated that based on the correlation between the 1 hour and 23 hour measurements and a heritability of 0.15, approximately 2000 animals needed screening to obtain estimates of genetic correlations with other production traits.

One-hour methane measurements, using portable accumulation chambers were recorded for 2600 animals at 5 INF sites – Cowra, Kirby, Rutherglen, Trangie and Katanning (Appendix 4; Robinson et al., 2011 (report for Sheep CRC, in prep for publication). Our results showed a small amount of genetic variation (heritability of 5%), which was lower than previous estimates and made estimating accurate genetic correlations impossible. Phenotypic correlations to other production traits are currently being estimated. More work is necessary to determine the best way to exploit the genetic variation that does exist. The screening has identified a number of issues that need to be explored further, including G x E variation, life-time emissions and relationships with feed intake, efficiency and liveweight. Identifying and studying outlying animals in more detail may provide some insight into potential mechanisms for reducing emissions. Possibilities include selecting animals with similar characteristics or breeding from identified outliers. At this stage, ASBVs are not considered a practical option.

Section 2. The physiology and rumen kinetics of high and low emitting animals

(Link to Appendix 5 and 6)

Twenty animals (10 highest and lowest methane-emitting animals from the 160 animals that were screened and validated through chambers and PAC 3 times in Glen Innes) were selected for the intensive physiology study (Appendix 5. Goopy et al., manuscript in prep). The 160 animals were a subset of the original 200 animals that were identified as being high and low emitters from the Sheep Genomics flock in the first large-scale screening (same animals discussed above). The 10 highest and lowest methane-emitting animals were placed in individual metabolism cages and dosed *per os* with solid and liquid phase digesta markers, and faeces and urine were collected for 6 d for later analysis. Rumen digesta profiles were measured by using ultrasonography, a novel and non-invasive approach to examining rumen parameters. Daily methane production was measured on all animals in individual respiration chambers for 24 h and a single rumen content sample was taken by stomach tube from each sheep. The data indicate that methane production is

affected by the time feed particles spend in the rumen (or the proportion of ingested feed retained in the rumen). The data also suggest that high methane emitting sheep have larger rumens than sheep with lower emissions, but that the proportion of rumen (organ) volume as gas is similar and that the dry matter percentage of the rumens is similar between high and low emitters. This is consistent with the notion that rate of passage of feed through the rumen is one factor contributing to systematic variation in methane production. The data are also consistent with the idea that this is in part due to intrinsic differences in rumen volume.

A complementary physiological study was also undertaken (Mark Barnett, PhD student) based on the positive correlation between methane yield and mean retention time (MRT) of digesta, which is known to be influenced by the hormone triiodothyronine (T3) (Appendix 6, Barnett et al., 2011). The hypothesis was that a decrease in the MRT in the rumen in response to administration of a T3 solution to sheep would reduce their methane production. 10 mature Merino wethers were injected with triiodothyronine (300 µg) on two different protocols (daily; n =5 and every second day; n=5) and the effect on daily methane yield, digesta mean retention times, dry matter digestibility (DMD), rumen VFA concentrations, microbial protein output and plasma T3 concentrations were studied. Compared to when injected with saline (control), injection of sheep with T3 every second day resulted in decreased methane yield ($P<0.05$) and lower acetate ($P<0.001$), butyrate ($P<0.001$) and propionate ($P<0.01$) concentrations in the rumen. Mean retention times of digesta, derived from faecal excretion of CoEDTA and Cr-mordanted fibre, were reduced in the total tract ($P<0.001$) and hindgut ($P<0.01$) but not in the rumen ($P>0.05$). DMD was not affected by injection of T3 every second day but water intake ($P<0.05$) and urine output ($P<0.01$) were increased. When sheep were injected with T3 daily, changes were only observed in plasma T3 concentration ($P<0.001$), digesta transit time ($P<0.05$) and volume of CO_2 produced ($P<0.05$). Our results indicate that increasing plasma concentration of the thyroid hormone T3 within physiological levels reduces digesta retention time, especially retention time in the hindgut and leads to a reduction in enteric methane yield. Further work is warranted to assess whether plasma T3 concentrations may be indicative of enteric methane yield.

Section 3. The microbial ecology of high and low emitting animals
(Link to BCCH1008 final report for detailed results and discussion)

Ninety-eight animals that were selected as being ‘high’ or ‘low’ based on the initial large scale PAC screening in WA were measured twice in respiration chambers to validate whether they remained ‘high’ or ‘low’ a year later. The animals were rumen sampled to provide data on VFA concentrations, pH, rumen ammonia and samples for molecular microbial ecology analyses. Samples were taken each time the animals went through the chambers so there are two samples for each animal. Rumen samples were also collected from the top and bottom 10 methane-emitting animals during the physiology and rumen kinetics experiment (section 2). Over 200 rumen sample were sent to both Dr Valeria Torok (SARDI; BCCH1008) and Dr Chris McSweeney (QLD) for microbial profiling and sequence analysis.

A preliminary analysis of the data indicated that there were no significant differences in microbial profiles (bacteria, methanogen, fungi, protozoa) based on their classification as 'high' or 'low' methane emitters or their actual daily methane production when measured in the chambers and corrected for liveweight, feed intake and feed intake. The subset of the samples analysed in greater detail using

pyrosequencing did not show any significant differences and it was decided that there would be no further benefit from analysing the other samples.

Section 4. Net feed intake and methane production
(Link to Appendix 7)

Since the beginning of May 2010 we have measured liveweight gain, intake, feed conversion ratio and methane emissions using the portable chambers on approximately 800 animals. Blood samples were taken to assess potential association between T3 (triiodothyronine) and methane production and yield as part of Mark Barnetts PhD studies. Rumen samples were also collected during NFI screening.
The analysis to date indicates that all traits differ significantly between sire groups. The differences between sires used in the maternal efficiency flock were: liveweight gain 45% (199 to 262 g/day); feed intake 35% (1.4 to 1.9 kg/day); feed conversion ratio 35% (5.7 to 8.2); estimated daily methane production 24% (66 to 82 L/day) and methane production adjusted for liveweight 27% (1.3 to 1.7 L/day per kg). Across these sire groups, daily methane production was related to liveweight (r = +0.71), liveweight gain (r = +0.57) and feed conversion ratio (r = -0.35), but not feed intake (animals were not on restricted intakes, which may explain why the relationship between intake and methane production was not significant). In all data sets there was a positive relationship between ASBV for YWT and daily methane production, but no other ASBVs are correlated with daily methane production and none are correlated with daily methane production adjusted for liveweight. The rumen samples have been collected and have been stored for analysis if we establish a relationship between NFI and methane production.

Section 5. Papers published from BCCH1015

Hegarty (2009). Livestock Breeding for Greenhouse Gas outcomes" http://www.livestockemissions.net/Portals/0/Publications/Animal%20variationWkshp/Report_part1.pdf

Goopy, J.P., **Hegarty, R.S.** and Robinson D. (2009). Two-hour chamber measurements provide a reliable estimate of daily methane production in sheep. p. 190 – 191. In Ruminant Physiology: Digestion, metabolism, and effects of nutrition on reproduction and welfare (Proceedings of the XIth International Symposium on Ruminant Physiology (Clermont-Ferrand, France)). Y. Chilliard, F. Glasser, Y. Faulconnier, F. Bocquier, I. Veissier and M. Doreau (ed.) Wageningen Academic Publishers, Wageningen.

Goopy, JP., Robinson, DL. Woodgate, R. and Hegarty RS. (2009). Repeatability of VFA concentration in sheep under field conditions. *Recent Advances in Animal Nutrition Australia.* 17:176

Robinson DL (2009) Improving the accuracy of selecting animals from reduced methane emissions. Assoc. Advmt. Anim. Breed. Genet. 18:64-647.

Robinson DL, Goopy JP, Hegarty RS, Vercoe PE (2010) Repeatability, Animal and Sire Variation in 1-hr Methane Emissions & Relationships with Rumen Volatile Fatty Acid (VFA) Concentrations. Proc. 9th Word Congress on Genetics Applied to Livestock Production, Leipzig, Germany, 1-6 August. Available at: http://www.kongressband.de/wcgalp2010/assets/pdf/0712.pdf

Robinson D.L., J. Goopy, R.S. Hegarty (2010) Can Rumen Methane Production be Predicted from Volatile Fatty Acid Concentration? Animal Production Science, 50: 630-636.

Hegarty RS, Alcock D, Robinson DL, Goopy JP and Vercoe PE (2010) Nutritional and flock management options to reduce methane output and methane per unit product from sheep enterprises. Animal Production Science 50:1026-1033

Bickell, S.L., Robinson, D.L., Toovey, A.F., Goopy, J.P., Hegarty, R.S., Revell, D.K., Vercoe, P.E. (2011) Four-week repeatability of daily and one hour methane production of mature merino wethers fed *ad libitum*. Association for the Advancement of Animal Breeding and Genetics, Australia, 19:415-418

Robinson, D.L., Bickell, S.L., Toovey, A.F., Revell, D.K., Vercoe, PE (2011) Factors affecting variability in feed intake of sheep with *ad libitum* access to feed and the relationship with daily methane production. Association for the Advancement of Animal Breeding and Genetics, Australia, 19:159-162

Goopy, J.P., Woodgate,R., Donaldson, A., Robinson, D.L., Hegarty, R.S. (2011) Validation of a short-term methane measurement using portable static chambers to estimate daily methane production in sheep. Animal Feed Science and Technology 166:219– 226

Section 6. Collaboration with other projects in the Reducing Emissions from Livestock Program

The main collaboration with other projects within the Reducing Emissions from Livestock Program has been BCCH.1008 led by Valeria Torock at SARDI (see final report for BCCH1008). We also had an obvious link to the beef-breeding project through Roger Hegarty, and that link was mainly focused on our interest in comparative biology. We have been very interested in comparing both the genetic parameter estimates as well as the microbial profiling data that we find in sheep and cattle. This collaboration has been *ad hoc* to this point because the programs are at slightly different stages but the intention is to make it a more formal arrangement in future proposals. We have also had a direct link to project BCCH1031, the demonstration site in Pingelly, WA, where some of the sheep that we have screened in the field and through chambers in WA that may be low emitters have been kept and used in grazing studies. Hegarty and Vercoe have also participated in field days at the other RELRP demonstration sites and presented on this work.

Outside the RELRP programme, we communicated and exchanged information with the 'breeding for lower methane production' MAFF-funded programme being run in New Zealand.

Section 7. Media coverage

List of radio interviews:

A list of radio interviews, TV and newspaper reports and field days provided below.

Walcha News, 17 September, p4
Esperance Express, 18 September, p12
Koondrook & Barham Bridge, 18 September, p6
Bendigo Advertiser, 19 September, p52

Country Leader Tamworth – insert, 21 September, p1
Daily News, 22 September, p14
Bainsdale Advertiser, 21 September, p6
Yass Tribune, 23 September, p18
ABC Radio Mt Isa, 22 September
Snowy River Mail, 23 September, p6
North Central News 23, September, p8
Rural Weekly insert, 25 September, p5
Warren Weekly, 23 September, p4
ABC Western Qld (Longreach), 25 September
ABC NE & NW (Tamworth), 25 September (2 clips)
ABC Darwin, 25 September (2 clips)
West Wimmera Advocate, 23 September, p12
The Land, 1 October, p5
Bombala Times, 7 October, p10
Farming Ahead, October 2009, p6
ABC South Coast WA (Albany), 21 October
Shepparton Adviser, 21 October, p22
ABC Midwest Wheatbelt (Geraldton), 23 October (2 clips)
ABC 2 (TV) National News Breakfast, 30 November
ABC1 (TV) Weekend News, Perth, Horbart, Darwin, Canberra, 30 November (4 clips)
ABC1 (TV) National Midday Report, 30 November
ABC1 (TV), Melbourne, Sydney evening news, 30 November (3 clips)
Southern Cross Rural News, 1 December (31 stations)
ABC Drive Melbourne, 2 December
The Land, 3 December p13
Sunday Herald Sun, 17 January, p30
Sunday Mail Adelaide, 17 January, p30
Sunday Mail Brisbane, 17 January, p17
Sunday Tasmanian, 17 January, p47
Sunday Territorian, 17 January, p9
Sunday Times Perth, 17 January, p29
2DU Dubbo, 18 January
2UE, 18 January
3AW (Melbourne), 18 January
4BC (Brisbane), 18 January
4BU (Bundaberg), 18 January
ABC 702, 19 January
NSW Statewide Drive, 19 January
Courier Mail, 23 January, p70
The Week, 29 January, p17
Ovine Observer #51, June pp. 1-3

Producer and Field Days, Open Days
UWA Open Day, 2009, 2010, 2011
Sheep CRC CCRP Producer Forum, Canberra May 2010
Dowerin Field Day, August 2010 and 2011
UWA Future Farm, October 2010 and 2011
CCRP Producer Forum, March, 2011
DAFF_MAFF combined workshop, Auckland, NZ, May 2011
UWA Special display in UWA Museum, June-July 2011
VicDPI Demonstration site field day, Hamilton, October 2011

Section 8. Appendices

Appendix 1. Goopy et al. (2009) - pdf provided with report
Appendix 2. Robinson et al. (2010) – pdf provided with report
Appendix 3. Goopy et al. (2011) – pdf provided with report
Appendix 4. Robinson et al. (2011) - report prepared for Sheep CRC and in preparation for peer review provided in full below
Appendix 5. Goopy et al. (201X) – manuscript in preparation and provided in full below
Appendix 6. Barnett et al. (2011) –pdf of Animal Production Science paper provided with report.
Appendix 7. Thompson et al. (2011) – paper in report for NFI provided in full below.

Appendix 4.
Genetic and phenotypic variation in methane emissions of the INF flock
Robinson, DL., Goopy,JP., Vercoe, PE., Oddy, VH., Hegarty, RS., Thompson, A. et al.

Executive Summary (~ 250 words max)

The results to date suggest a small amount of genetic (breed/sire) variation. More work is, however, desirable to determine the best way to exploit this variation. Issues that need to be explored further include G x E variation, life-time emissions and relationships with feed intake, efficiency and liveweight. Identifying and studying outlying animals may provide some insight into potential mechanisms for reducing emissions. Possibilities include selecting animals with similar characteristics, or breeding from identified outliers. At this stage, with current measurement protocols, ASBVs may not be a practical option.

Introduction

To date, there have been limited investigations into the heritability of methane emissions, usually involving small numbers of often genetically diverse animals and an evolving suite of measurement protocols. For example, in one of the larger studies conducted to date, Robinson et al., (2010) reported an estimated heritability of 13.1% for 1-hr methane emissions (adjusted for liveweight) of 708 non-pregnant ewes in the Sheep Genomics flock at Deniliquin. These results were based on tests of batches of 15 animals, carried out at approximately 1.5 hr intervals, over the course of 11 days. The sheep were fasted overnight until 2 hours before the start of measurement, when they were allowed access to feed for 1 hour, followed by an hour to allow the rumen contents to start fermenting before the 1-hour measurement period commenced.

When the 100 highest and 100 lowest emitters were transported to Glen Innes and re-tested, animal effects remained, but not the previously-observed effects related to the animals' sires. Substantial variation was noted in the amount of feed eaten in the hour before measurement, leading to concerns that the test procedure might be increasing the variation in measured methane emissions, making it unrepresentative of an animal's normal behaviour. Measurements were therefore made on the same animals using a modified test, in which sheep were measured directly off-pasture. In these studies the amount of feed consumed prior to the test was unknown.

Results from the modified test (immediately off pasture) seemed promising in that large differences between sires were identified. In addition, for the first 40 animals tested in respiration chambers, the modified (off pasture) test was more highly correlated with respiration chamber measurements in which feed intake was controlled to be a fixed proportion of liveweight. Before adjusting off-pasture data for liveweight and adjusting respiration chamber measurements for feed intake, the correlation for the first 40 animals was 0.48. The correlation of off-pasture emissions adjusted for liveweight and respiration chamber measurements adjusted for feed intake was 0.26. For the previous test involving an overnight fast, correlations with respiration chamber measurements were 0.15 or less. Subsequent results, based on respiration chamber measurements of 160 animals over the following months (being written up for a journal paper) also suggest that the off-pasture test is somewhat more closely related to respiration chamber measurements than the previous protocol.

Although it would have been useful to assess the long-term repeatability and consistency over time of alternative test protocols before measuring Information Nucleus Flock animals, it was not possible to do this and meet the requirement to screen large number of sheep within the specified timeframe. The screening was therefore carried out using the unvalidated off-pasture test.

Objectives

The objective was to screen sheep in the IN flock for methane emissions and to determine the heritability of the trait.

Materials and methods

Data. One-hour methane measurements, using portable accumulation chambers (PAC,Goopy et al., 2011) were recorded at 5 INF sites Rutherglen, Trangie and Katanning. At Cowra, Kirby, Rutherglen and Trangie, there were 6 measurement sessions per day, starting at approximately 8:10, 9:40, and 11:10 am, 12:40, 14:10 and 15:40. Individual observations, site means (solid lines) ± SE (dotted lines) are shown in Fig1 below for CH_4 emissions (dL/hr) and in Fig 2 for CO_2 (%), after the carrying out the data checks described in the next section.

Fig 1. Individual observations, means (solid lines) ± SD (dotted lines) for 1-hr CH_4 emissions (dL) at Cowra, Kirby, Rutherglen and Trangie.

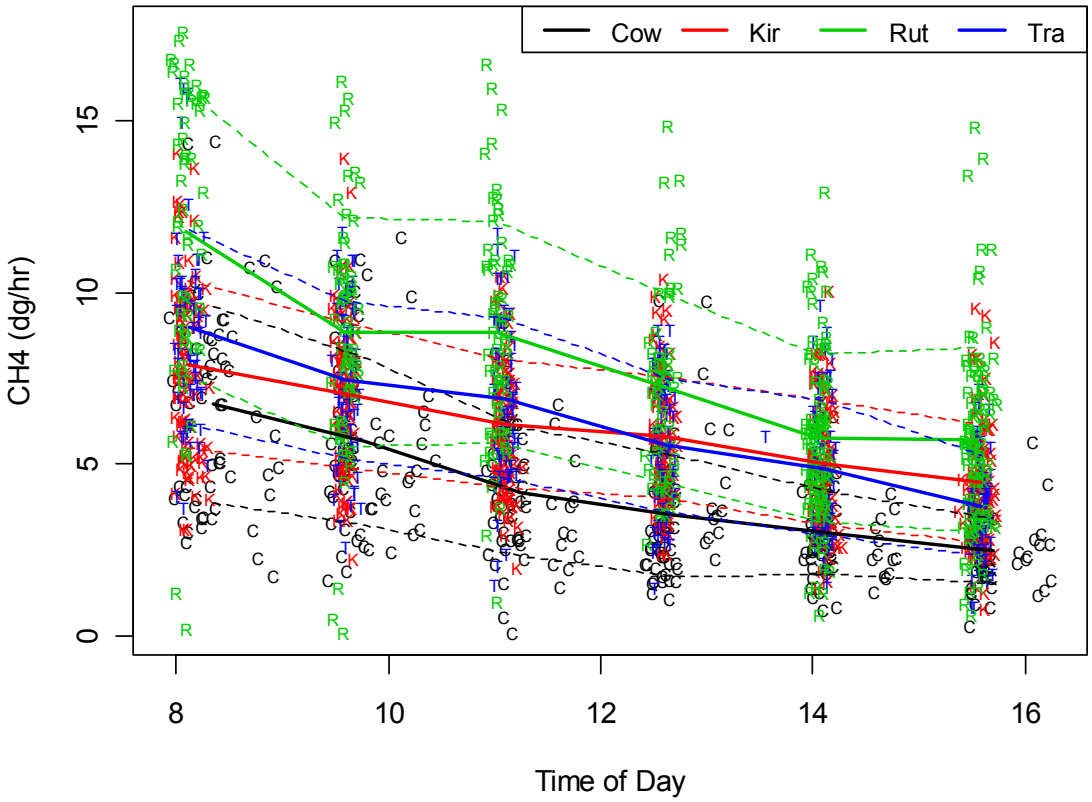


Fig 2. Individual observations, means (solid lines) ± SD (dotted lines) for 1-hr CO_2 concentrations (%) Cowra, Kirby, Rutherglen and Trangie.

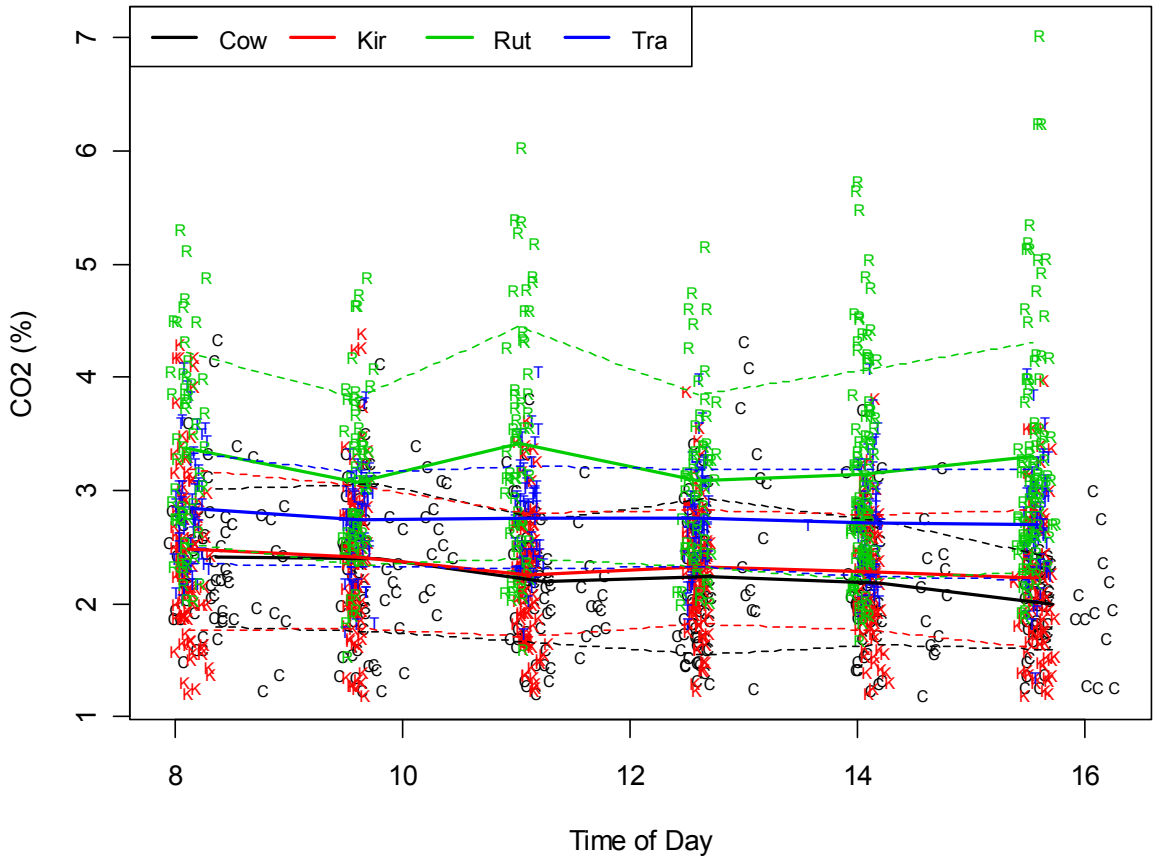
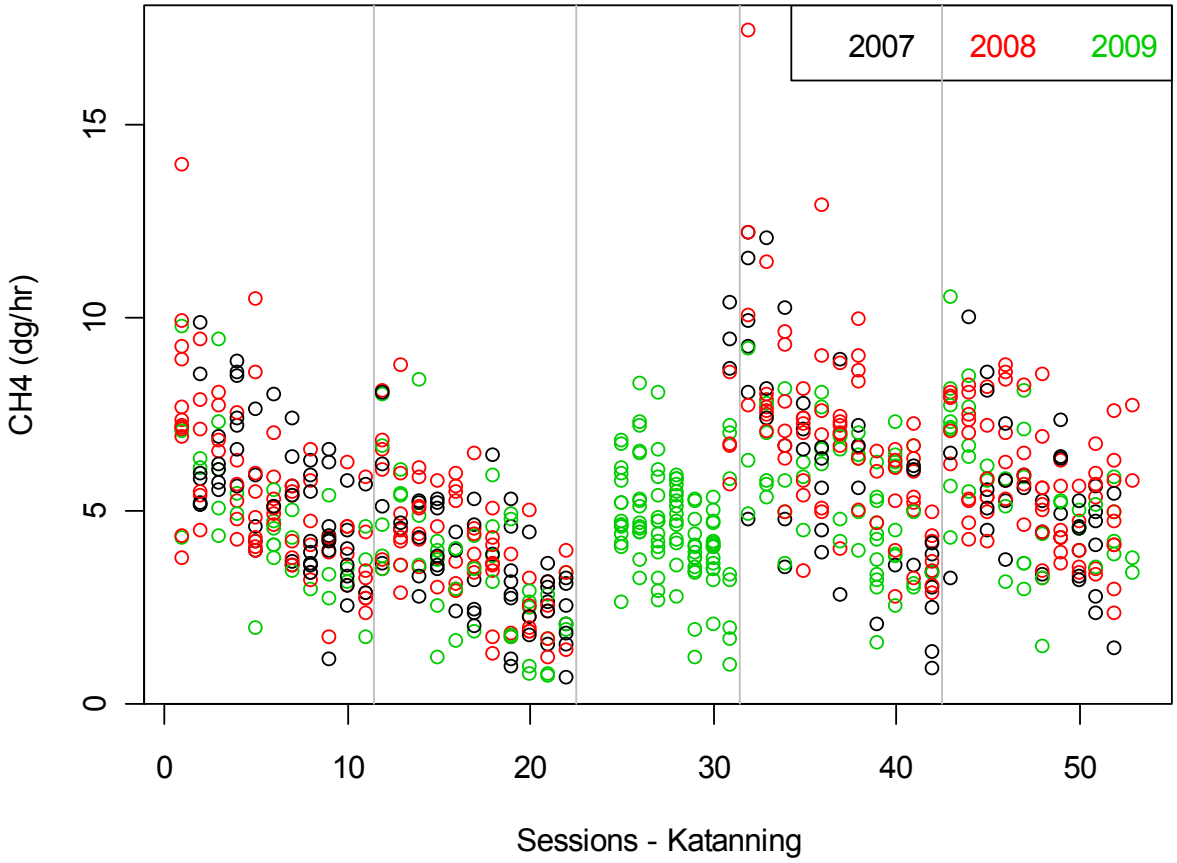

Cow
Kir
Rut
Tra
CO2 (%)
1
2
3
4
5
6
7
8
10
12
14
16
Time of Day

Fig 3. Individual observations, by birth year and session number, Katanning, WA. Katanning had 11 sessions on days 1, 2, 4 and 5, with tests conducted from about 6 am to 6:30 pm and 7 sessions on day 3 from 8:40 to 16:30 (Fig 3). Individual days are separated by grey vertical lines. Colour indicates year of birth. Katanning did not record CO_2. The unusually high readings on the last session of day 3 appear genuine, so retained in the analysis.

Dates of testing are shown in the table below.



Site	Start Date	End Date	Test Days	Comments
Cowra	29/11/2010	3/12/2010	5	Test on all days
Kirby	15/02/2011	24/02/2011	8	No tests 19, 20 Feb
Rutherglen	31/01/2011	7/02/2011	7	No tests 6 Feb
Trangie	13/12/2010	16/12/2010	4	Tests on all days
Katanning	22/11/2010	30/11/2010	5	No tests 25, 26, 27, 28

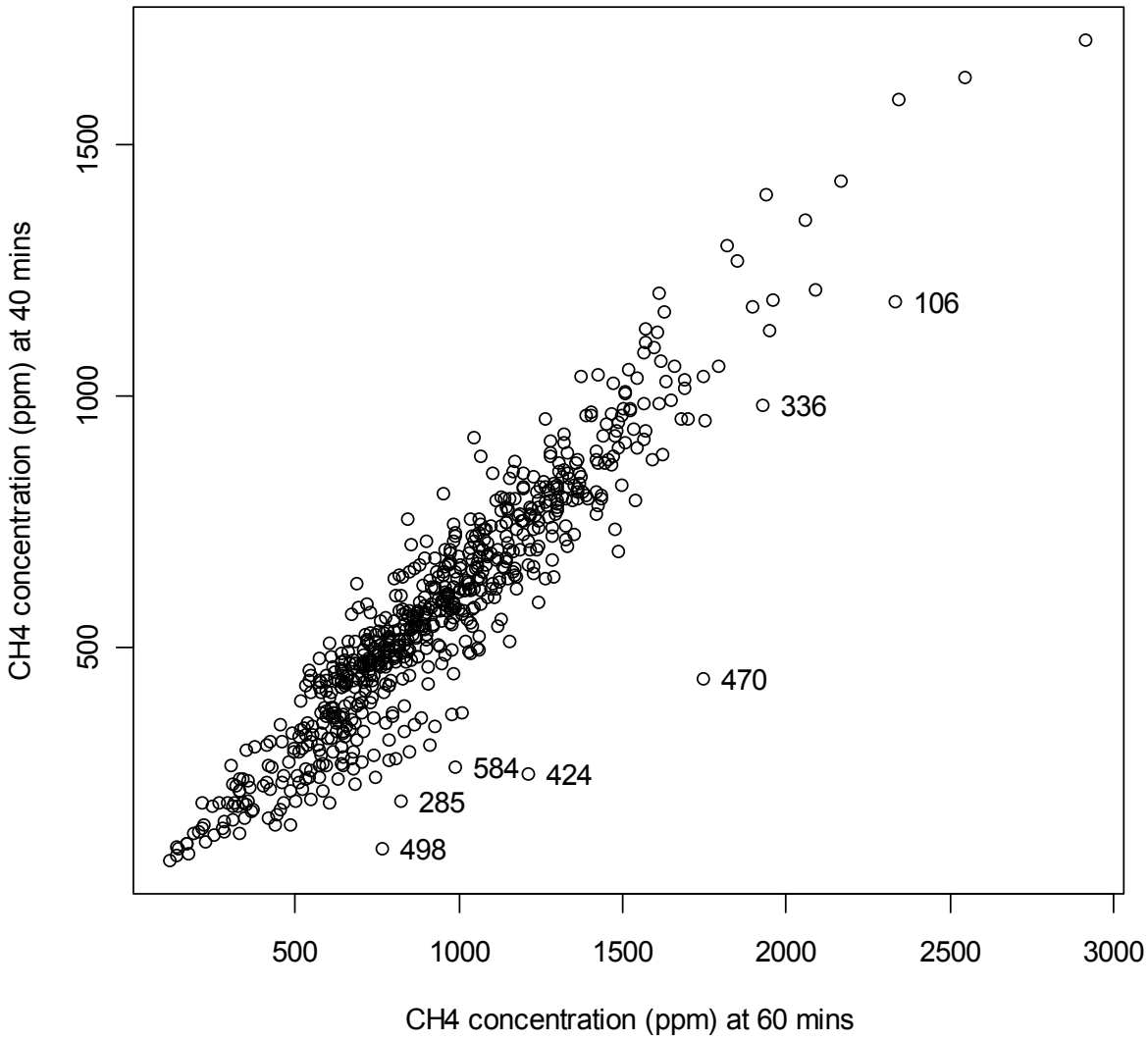
Data Checking

Basic data checks included omitting measurements for:

1) sheep with missing identify tags
2) animals that were sick, flystruck or lame
3) had agitated movements that might have breached the seal of the PAC.

At Cowra, Kirby, Rutherglen and Trangie, records with unrealistically low CO_2 measurements (less than 1.2%) were also deleted. At Katanning, CO_2 was not recorded, but methane concentrations were measured after 20 and 40 minutes in the PAC as well as after 60 minutes, allowing the build-up of methane over time to be examined. A total of 7 outliers, identified graphically by linear regression, were removed (Fig 4) as well as one record with an incorrectly-recorded liveweight.

Fig 4. Relationship between CH_4 concentration at 40 and 60 minutes. Numbered outliers were deleted



An additional 28 animals that could not be found in the pedigree file, or had unknown sires were also omitted. Numbers of animals before and after data checks are shown in Table 1.

Table 1. Total Records, number removed during validation, number analysed

	Records	Sick/lame Agitated	No ID	Outlier/ low CO_2	Unknown pedigree	Unknown sire	Used
Cowra	412	3	1	32		3	373
Kirby	619	1	1	13	1	12	591
Rutherglen	447	9	1	1	2		434
Trangie	369	7			1	1	360
Katanning	753	14		7		8	723
	2600	34	3	54	4	24	2481

Table 2 shows the number of animals with 1, 2 or 3 methane records, and the number of dams by number of offspring with methane measurements. Table 3 shows the number of dams with offspring in 1, 2 or 3 different years (i.e. had measured offspring born in 1, 2 or 3 of the years 2007, 2008 and 2009).

Table 4 shows the number sires by number of offspring, and by the number of sites at which the sire was used, e.g. 34 sires were used at only 1 site. The remaining 155 sires were used a more than 1 site, with 49 sires being used at 4 out of the 5 sites and 7 sires at all 5 sites. Only 10 of the 189 sires were used in more than 1 year; 16% of dams were used

in more than 1 year. The mean number of offspring per sire was 12.2 and the mean number of offspring per dam 1.4.

Table 2. Number of animals by no of records; numbers of dams by number of offspring

	No of animals by no of records				Number of dams by no of offspring					
	1	2	3	All	1	2	3	4	5	All
Cowra	346	6	5	357	151	63	19	3	1	237
Kirby	646	28	7	681	426	84	17	5	0	532
Ruthergle n	511	40	0	551	337	73	19	2	0	431
Trangie	298	68	0	366	132	63	24	5	3	227
Katanning	336	12	0	348	129	71	24	1	0	225
All	2137	154	12	2303	1175	354	103	16	4	1652

Table 3. Number of dams used for 1, 2 or 3 years

	Number of dams used for 1, 2 or 3 years			
	1	2	3	All
Cowra	197	33	7	237
Katanning	482	48	2	532
Kirby	365	59	7	431
Ruthergle	163	49	15	227
Trangie	175	50	0	225
All	1382	239	31	1652

Table 4. Number of sires by number of offspring and number of sites where it was used

Offspring	1-4	5-8	9-12	13-16	17-20	21-24	25-28	29-32	35	Total
No of sires	27	37	33	45	21	12	11	2	1	189

Number of sites	1	2	3	4	5	Total
Number of sires used at the above number of sites	34	24	75	49	7	189

Statistical Analysis

An initial exploratory analysis was conducted by fitting sire and dam models to the data from each individual site (Cowra, Katanning, Kirby, Rutherglen, Trangie) to identify the most important factors affecting measurements at each individual site. Factors considered included time of measurement (hours since the first measurement of the day) as a linear and quadratic effect, test day and session, breed, year of birth and other relevant information e.g. at one site some sheep were shorn and others were woolly.

All sites were then combined into a single dataset and analyses conducted of both the untransformed data (CH_4, dL/hr) and after a logarithmic transformation ($LTCH_4$) to overcome the skewness (Equation 1, Table 5 and Fig 5).

$$LTCH_4 = 10*\ln(CH_4(dL/hr) + 1) \quad (1)$$

Log transformations are appropriate when the effects are expected to be multiplicative, or proportional mean, so if 10% difference between animals is observed when the mean is 5 (i.e. 5 ± 0.5), when the mean increases to 10, we expect the same 10% difference (10 ± 1.0), rather than the same absolute variance (10 ± 0.5) that would be expected under the normal model for untransformed data.

After transformation, the most variable site (Rutherglen) was only twice as variable as the least, compared to 3 times as variable for the untransformed data (Table 5). Even

after transformation, modelling showing considerable differences in residual variances for the different sites ($P<10^{-16}$), so the basic model included separate terms for the residual variances at each site.

Table 5. Means, minima, maxima and variances of CH_4 emissions and liveweight

	Untransformed CH_4 emissions, dg/hr				Log-transformed data (Equation 1)			
Flock	Mean	Min	Max	Var	Mean	Min	Max	Var
Cowra	4.3	0.2	14.5	6.0	15.7	1.5	27.4	19.0
Katanning	5.1	0.7	17.4	4.4	17.5	5.0	29.1	12.8
Kirby	6.1	0.9	14.1	5.2	19.0	6.2	27.1	11.0
Ruthergle n	7.6	0.1	17.6	13.4	20.5	1.3	29.2	21.9
Trangie	6.3	0.9	16.1	7.6	19.1	6.6	28.4	15.8

	Liveweight (kg)			
	Mean	Min	Max	Var
Cowra	71.3	43.4	109.5	117.3
Katannin g	53.8	35.5	83.5	60.0
Kirby	46.2	30.2	75.2	47.6
Ruthergle n	53.2	30.4	88.8	112.2
Trangie	66.9	40.2	100.5	84.8
Mean	58.3	35.9	91.5	84.4

Wald tests were used to assess the significance of terms in the fixed effects model. The significance of random terms was assessed using likelihood ratios. The final model included fixed effects for flock, and covariates for time of measurement (**tim**) and liveweight (**lwt**). Both covariates were standardised to have mean zero, with different slopes fitted for each of the 5 flocks, all of which were positive, implying that heavier animals emit significantly more methane. However, as can be seen from Table 5, this relationship does not hold across sites. The dominant factor when comparing sites is feed quantity and availability at each site, so that in this case the site with the heaviest animals (Cowra) had the lowest average methane emissions.

For untransformed data, the analysis also included an overall quadratic term for time of measurement; this term was not significant and so not fitted in the analysis of log-transformed data. There was no effect of birth or rearing type (single, twin or multiple), sex, nor age of dam, so these effects were not fitted.

Significant random effects included breed type (**brdx:** MM, MATM, TM and TMAT or unknown (n=5)), year of birth (**drop** = 2007, 2008 or 2009), drop.flock, day of measurement (within flock), session number (within day and flock) and PAC number (within flock). Katanning sheep had more detailed breed information, with animals classified into 14 breed types. The effects of breed type at Katanning appeared to be highly significant, so was retained in the model. In contrast, numeric 2-digit codes for sire and dam breeds, extracted from the Sheep CRC database, explained no variation, so were not included in the final model. There was also no effect of contemporary group within flock.

The final model was therefore:

CH_4 = flock + flock.tim + flock.lwt + animal_terms + brdx + drop + flock.drop + flock.PAC + flock.day + Breed (Katanning sheep only) (2)

The above model was fitted using a range of different animal_terms, whose likelihoods were compared using the likelihood ratio test. Included were

anim_gen (**AG**, animal genetic effect, fitted using all available pedigree information - parents, grandparents etc)

sire (**S**, effect of the animal's sire)

dam (**D,** permanent environmental effect of the animal's dam)

anim_phen (**AP**, phenotypic effect of the animal)

Fitting the above terms singly and in combination allowed the most likely model to be determined, and the significance of sire, dam and animal genetic effects to be assessed.

Results

For the analysis of *liveweight*, the most likely animal model was: AG + AP, showing that use of genetic relationships from the pedigree file provided a better fit than assuming that sires and dams were unrelated. In these relatively mature animals, no permanent environmental effect of the dam was detected. Significant fixed effects included flock, birth type and the interaction of flock and time of measurement. For flocks that weighed animals on exit or entry to the PAC, this represents the effect of the time spent in the holding area with limited access to feed, leading to reductions in gut fill. Rearing type (P=0.11) and sire type (P=0.24) were not quite significant, but retained in the model to avoid potential problems that might arise from confounding with these effects. The estimated heritability of liveweight from this model was quite high - 56%.

I n contrast, estimates of animal effects for *methane* emissions were much lower. Table 6 compares estimates of variance components from fitting model S + D + AP and model

AG + D + AP. In order to estimate heritability, a mean residual variance was calculated as:

MR = mean (RKa, RC, RKi, RT, RR)

where RKa, RC, RKi, RT and RR were estimated residual variances at Katanning, Cowra, Kirby, Trangie and Rutherglen respectively. Estimates of heritability were calculated as:

Model S + D + AP: est-h^2 = 4*Var(S)/(Var(S)+Var(D)+Var(AP)+MR)

Model AG + D + AP: est-h^2 = Var(AG)/(Var(AG)+Var(D)+Var(AP)+MR)

Table 6. Estimated variances of log-transformed data ($LTCH_4$, models S + D + AP and AG + D + SP) and untransformed data (CH_4, model S + D + AP)

Model	$LTCH_4$: S + D + AP		$LTCH_4$: AG + D + AP		CH_4 (dL/hr) S + D + AP	
Term	Estimated Variance	*SE*	Estimated Variance	*SE*	Estimated Variance	*SE*
Sire/AG	0.095	0.066	0.321	0.267	0.039	0.027
Dam (D)	0.296	0.260	0.263	0.264	0.110	0.103
Animal phenotypic(AP)	1.044	0.495	0.934	0.505	0.382	0.209
flock	0.070	0.061	0.066	0.060	0.117	0.044
breed	0.123	0.170	0.133	0.206	0.061	0.083
Drop (2007, 08 or 09)	0.188	0.254	0.236	0.305	0.064	0.091
Drop (within flock)	0.208	0.147	0.220	0.156	0.084	0.061
Katanning Breeds	1.482	0.459	1.486	0.460	0.597	0.188
Test day (within flock)	1.347	0.849	1.268	0.824	0.209	0.170
PAC (within flock)	0.718	0.169	0.715	0.168	0.279	0.067
Resid-Katanning (RKa)	4.595	0.525	4.639	0.528	1.561	0.211
Resid-Cowra (RC)	7.666	0.822	7.705	0.825	2.548	0.297
Resid-Kirby (RKi)	3.328	0.470	3.369	0.474	1.683	0.217
Resid-Trangie (RT)	5.766	0.701	5.838	0.704	2.974	0.333

Resid-Rutherglen (RR)	13.756	1.117	13.743	1.116	7.218	0.566
Estimated heritability	4.6%		3.7%		4.2%	

The significance of animal effects is best assessed using the likelihood ratio test. Two tests were carried out.

1) the P-value for dropping individual terms, e.g. the P-value for dropping term AP compares the likelihood of fitting all terms in Table 6, with the likelihood of the model containing all terms except AP.

2) the P-value for adding in a single animal term, i.e. the likelihood of comparing model 2) with no animal terms with the same model containing a single animal term (AG, S, D or AP)

Table 8. P values for adding/dropping animal terms for $LTCH_4$ (model 2)

term	AG	S	D	AP
P for adding	0.008	0.072	0.007	0.001
P for dropping	0.130	0.103	0.261	0.023

The results for $LTCH_4$ (Table 8) show a clear and significant animal phenotypic effect, but that there is insufficient information to clearly separate the animal effect into additive genetic, sire or dam effects, although the small number of repeat tests provided enough information to separate animal phenotypic effects from the residual variation.

For $LTCH_4$, the differences between the likelihoods of models S + D + AP and AG + D + AP was 0.18, implying that both were fairly similar, with the former slightly more likely. Unlike the analysis of liveweight (where the most likely model was AG+AP), use of genetic relationships from the pedigree file did not improve the fit.

The total genetic and phenotypic animal variation for $LTCH_4$ (0.095+0.296+1.044) = 1.435 is almost half the residual variation at the least variable site (Kirby). For the untransformed data, the total (0.531 dg/hr), provides some indication of the repeatability on the untransformed scale. Using the formula: rpt = Animal/(Animal + Residual), at the least variable site (Katanning), rpt = 1.07/(1.07+3.056) = 25%. For traits with low repeatability, a new trait calculated as the mean of two or more measurements at each site would be more accurate, and perhaps have double the heritability estimated here (Table 6) for a single measurement.

Fitted values for the effect of year of birth revealed that the youngest (2009-born) sheep appeared to emit less methane than would be expected of older animals with similar liveweight. As shown in Table 8, 2009-born animals were, on average, 7.2% lighter than 2007- and 2008-born animals. The model used in this analysis allowed for the significant differences in the slope of the relationship with liveweight between sites, but assumed the relationship was the same for the different breeds and different birth years. These assumptions need to be fully tested before firm conclusions can be drawn.

Table 9. Estimated means for liveweight and $LTCH_4$ by year

Birth year	2007	2008	2009	Ave SED
$LTCH_4$	25.7	25.7	25.2	0.14
liveweight	62.6	61.1	57.4	0.92

A curious factor of this analysis was the large and significant breed effects at Katanning, but not elsewhere. Much of the effect was due to 10 East-Friesian sheep that had much lower CH_4 emissions than expected for their liveweight. The three main breeds at Katanning were Merino (313 animals), Poll Merino (153) and Border Leicester (159), with another 108 sheep distributed over 11 other categories. After adjustment for liveweight, emissions of the three main breeds were almost identical.

The variation due to breed types at Katanning may therefore be due some unusual animals, or differences in the relationship with liveweight for different breeds. Such differences (in the relationship of liveweight and CH_4 emissions for different sire types) were noted in measurements recorded at Glen Innes for the Sheep GENOMICS animals. Investigation of this phenomenon is ongoing.

The relationship of a potential methane trait with other measured traits was examined by calculating correlations between predicted sire means from model S + D + AP with ASBVs for 104 Merino and 1 Maternal sire.

Correlations of predicted sire means for $LTCH_4$ with selected ASBVs

bwt	ywt	awt	cwt	cfat	yemd	cemd	agfw	acfw	afd	adcv
0.11	0.23	0.21	0.20	0.00	0.01	0.03	-0.02	-0.04	0.04	0.11

bwt, ywt, awt, cwt =birth weaning, adult and carcass weight; cfat = carcass fat, yemd, cemd = yearling and carcass eye muscle depth; agfw, acfw = adult greasy and clean fleece weight; afd, adcv = adult fibre diameter and CV. (NB abbreviations have not been confirmed)

As shown in the above table, despite the adjustment for liveweight in model 2, some residual correlation with weight ASBVs remains.

Discussion

The analysis reported here should be considered preliminary in that the relationship of methane emissions with liveweight and CO_2 has not yet been fully explored. The results to date show a small amount of genetic variation. Consequently, investigation of 'unusual' animals may lead to greater scientific insight which could prove invaluable in assessing the best options for reducing methane emissions. Intriguing coincidences are also worth investigating, e.g. the lowest-emitting sire from the new off-pasture test of the Sheep Genomics animals at Glen Innes was an East Friesian, and emissions of 10 East Friesian sheep were also noted as outlying at Katanning.

One of the most challenging aspects of methane research is variation over time, which has led to inconsistent and contradictory results. Even for data collected at a single site, where all sheep experience the same management and feeding conditions, estimated heritability was 13.1% after adjusting for liveweight (Robinson et al., 2010). Because of the additional G x E variation usually found in data covering a rage of sites and nutritional levels, a lower heritability would be expected.

This analysis revealed significant animal variation in emissions (P=0.001), some of which appeared to be genetic, although likelihood ratio tests were not able to separate animal and sire effects at a probability level less than 0.05. Conditions at the test sites varied substantially, e.g. the site with the heaviest animals (Cowra) had the lowest average methane emissions. This is likely to have led to G x E variation and additional difficulties separating sire and animal effects.

Although the estimate of heritability is modest, it could nonetheless be worthwhile. When residual variation is high, heritability is low, unless repeat measurements are taken on the same animals. A more useful statistic is the estimated sire variation, which, from the analysis of untransformed data, implied a genetic SD equal to 6.7% of the mean. Selecting sires in the top 50% for methane emissions (which should be possible without materially affecting the value of the selection index for other traits) would therefore result in a 2.7% reduction in methane emissions of the offspring, and potentially greater reductions if some selection pressure could also be applied to dams. A cost-benefit analysis could determine the desirability of such a strategy.

The difference between animals was clear and significant. The SD of total variation between animal was 12% of the mean. This is the variation of animals under the conditions in which they were tested. If would be worthwhile and of interest to monitor this variation over time in a commercially-relevant flock, to determine the repeatability over time and the extent to which animals re-rank under different conditions. This might require testing the same animals 3 or 4 times over the course of a year, with all animal being measured over two short periods (1 hour or less) each time they are tested.

The high correlation noted between methane concentrations at 40 minutes and 1 hr suggests that the 1-hour test period could be shortened, allowing more animals to be tested per day. Alternatively, if the same number of animals is tested per day, the reduction in the time off feed for animals tested in the later (e.g. 5th and 6th sessions) could increase the accuracy of comparing animals. Because of the high residual variation, it is desirable for all animals to be tested twice, so that estimates of animal effects at the different times of year are based on the mean of two independent measurements. Measuring all animals twice (even for a shorter time period e.g. 40 instead of 60 minutes) should also provide more accurate estimates of day, time of day, test period and PAC effects, again improving the overall accuracy of testing.

Results from the previous test protocol, involving an overnight fast then access to feed for the period from 2 to 1 hour before measurement in the PAC, showed that differences between 'high' and 'low' methane groups remain significant after 4 months despiter feeding conditions that led a doubling of average methane emissions. The differences between high and low groups remained significant, even after adjustment for feed intake in the hour before measurement (Goopy et al., in preparation).

A series of repeat tests at 1 or 2 INF sites, sampling the different pasture quality and availability over the course of a year, could therefore shed considerable light on methane emissions and the repeatability of animal effects. The protocol of two (possibly shorter) measurements per animal at each test – and 3 tests over the course of a year (a total of 6 measurements per animal) – would result in much lower residual variation, so that animals with consistently lower emissions from a commercially-relevant flock can be indentified and studied. A combined analysis of the initial measurements discussed in this report, together with the proposed follow-up measurements, should facilitate the separation of animal phenotypic and genetic effects, as well as identifying extreme animals for further study, perhaps in conjunction with the 'Lifetime Methane' project. The results will also enable a much better estimate of heritability and, if heritable, genetic correlations.

Variation in methane emissions over time is noted in the literature. For example, for sheep on a diet of molassed perennial ryegrass silage, fed at 1.3 × maintenance metabolisable energy requirements, repeatability of methane yield from 1-day respiration chamber measurements was 0.16, after a 13-15 day interval (Pinares-Patiño et al., 2011). This represent an upper bound to the heritability of methane emissions based on a single respiration chamber measurement under controlled dietary conditions. If animals are tested at different locations, with different dietary conditions at each location, a lower heritability value might be expected, because of additional G x E variation.

G x E has also been noted in beef cattle. For example, when grazing high quality pasture, low residual feed intake (RFI) cows had lower CH_4 emissions per kg liveweight of cows and their calves (if present), but there were no differences in emissions on low quality pasture (Jones et al., 2011). When molecular microbial profiling techniques were used to investigate rumen microbial composition, diet was found to significantly alter all microbial communities. Moreover, significantly different archaeal and methanogenic communities for high and low RFI cows were found only when the cattle were fed high quality pasture (Torok et al., 2011).

The difficulties of obtaining consistent results was also noted by Herd et al., (2011), who compared methane yield, measured using SF_6, of 6 sires and their male offspring (average 7.7 progeny per sire) and 7 sires and their female offspring (average 12.2 progeny per sire). In both cases the correlations were negative (r=-0.53; P=0.22 for the heifers and r=-0.30; P=0.57 for the bulls). Although neither correlation was significantly different from zero, this example aptly illustrates the challenges to be faced identifying circumstances and experimental protocols under which methane emissions are likely to have the highest repeatability and heritability, so that they can be usefully exploited.

Given the existence of apparently large G x E variation, it is important to carry out repeated tests on the same animals under the range of conditions they are likely to experience. As noted above, this could be achieved by a series of repeat tests at 1 or 2 INF sites, sampling the different pasture quality and availability over the course of a year

Conclusions

The results to date show a small amount of genetic (breed and sire) variation. More work will, however, be required to determine the best way to exploit this variation. Issues that need to be explored further include estimates of longer-term repeatability over the course of a year, G x E variation, life-time emissions and relationships with feed intake, efficiency and liveweight. Identifying and studying outlying animals may provide some insight into potential mechanisms for reducing emissions. Possibilities include selecting animals with similar characteristics, or breeding from identified outliers. At this stage, it is too early to consider development of ASBVs for methane emissions.

Proposed Publications

Suggested publication:
Genetic and phenotypic variation in methane emissions of Australian sheep[1]
D.L. Robinson, J.P. Goopy, R.S. Hegarty, P.E. Vercoe et al.
Other aspects of this work, e.g. modelling the relationships with CO_2 or with liveweight may also be considers as possible future papers.

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Appendix 5.

Low methane producing sheep exhibit different gut kinetics

J.G. Goopy, R.S. Hegarty, D. Tucker, A. Donaldson, F.E.M. Haynes and V.H. Oddy

Introduction Australia and others are devoting considerable resources to the amelioration of enteric methane production by livestock. In the Australian context of predominantly extensive pastoral production, the most practicable strategy appears to be that of exploiting observed differences in methane production within the ruminant populations (Blaxter and Clapperton, 1965, Pinares-Patino *et al* 2003a, Goopy *et al* 2006). Although unknown, it may be deduced that such observed differences arise from one or more of the following causes: a) less organic matter (feed) being fermented in the rumen; b) a shift in VFA production toward H^+ utilizing (propionate or reductive acetogenisis) pathways; c) an increase in the proportion of microbial cells produced from fermentation.

Alterations in rumen mean residence (or turnover time) have the ability to affect both extent of degradation of organic matter in the rumen, and the amount of undegraded microbial matter which passes post-ruminally, and as such is a prime candidate for the accounting of observed differences in methane production between animals fed a constant diet. Further, earlier work of Pinares-Patino *et al* (2003b), suggested that up to 40% of the observed variation in methane production in sheep could be attributed to differences in mean rumen outflow.

We measured Daily Methane Production (DMP) in 160 mature ewes and selected 20 animals, extreme for the trait of DMP/DMI for further investigations. We hypothesised that differences in methane production/unit food intake would be reflected in physical differences in the rumen environment.

Materials and Methods

All animal procedures were carried out under ACEC approval number UNE 09/144

Selection of sheep and measurement of methane emissions.
Of sheep originally selected from the SheepGENOMICS flock for estimation of daily methane production (Goopy *et al*, submitted), 160 animals had DMP assessed three times by open-circuit calorimetry, while consuming a ration of a 50/50 mix of lucerne and oaten chaff fixed at 120% of maintenance requirements (Goopy *et al,* in preparation). Sheep were ranked by average emissions (CH_4 g/DMI kg), and 10ea high and low emitting animals (>±1SD) selected for further study. Selected ewes were housed in individual pens and adapted to the same diet as previously for 14d, fed as a single morning meal with refusals removed and recorded each day. At the conclusion of the adaption period, enteric CH_4 production was measured over a 22 h period (Day 0) for each sheep, as described by Bird *et al*. (2008). Briefly, CH4 production was calculated as chamber air flow multiplied by [CH_4] in the chamber adjusted for [CH_4] of the incoming air and temperature and atmospheric pressure in the chamber. The 22h value was converted to DMP by multiplying by 24/22. Air flow though each chamber (mean = 98.8 L/min) was measured using an AL800 dry gas meter (American Metering Company, Nebraska City, NE, USA). The [CH_4] (ppmv) was measured in chamber incoming and exhaust air streams using an Innova 1312 Multigas Analyser (California Analytical Instruments, Orange, CA, USA) calibrated for CH_4, CO_2 and water vapour. Air temperature, relative humidity and absolute gas pressure were measured by Easysense sensors (part nos. 1113201, 113220, 113264; Serrata Pty Ltd., Sydney, Australia) and recorded using Sensing Science Laboratory software (Data Harvest Group,

Bedfordshire, UK). Feed refusals were measured at the conclusion of the 22h CH_4 measurement period; animals returned to pens, fed, then measured a second time (Day 2) after a day's respite.
Sheep were then transferred to metabolism crates directly after the second measurement for the conduct of digesta kinetic studies (6d) and fed as described above. At the conclusion of the collection period (described below) all animals had enteric methane production measured a further two times.

Measurement of digestibility and rumen kinetic parameters.
The 20 ewes were confined in metabolism crates and offered feed as above (1.2x calculated maintenance requirement). Immediately prior to confinement in the met crates, each animal was dosed *per os* feed mordanted with Cr (5g feed; total dose of Cr = 211.8mg) and with Co-EDTA (45ml; total dose = 1,235mg Co) (Uden et al, 1980). Total collection of faeces and urine were made as follows. Faeces were collected 8h after dosing, then at 2h intervals until 24h, 4h intervals until 72h, then at 8h intervals until 96h then 12h intervals until 140h post dose. Total faeces were collected, weighed and a subsample taken and dried to constant weight at 80^{o}C then stored at room temperature. Total daily excretion of urine was collected into buckets to which 100mL 10% HCl was added and sampled once daily (1000h). Volume was recorded, and then made up to 3l volume and a 150ml subsample taken. Samples were stored at -20degC until analysis. Apparent DMD was calculated by subtracting total faecal dry matter from total dry matter of feed consumed over 6d.

Faecal samples were subjected to a modified Sealed Chamber Digestion (Anderson and Henderson, 1986) to remove organic matter. Duplicate aliquots (0.2 ± 0.01 g) of each of the dried and ground faeces samples were placed in 100 ml Schott bottles with two ml of freshly prepared 7:3 (v/v) mixture of perchloric acid ($HClO_4$) and hydrogen peroxide (H_2O_2). The samples were allowed to stand overnight, then a further 1 ml of H_2O_2 was added, the bottles sealed and placed in an oven set at 80^{o}C for 30 minutes. This was repeated, except the bottles were placed in the oven for 1 h. Samples were equilibrated gravimetrically (to 25 g) after cooling by addition of distilled water, shaken and then filtered (Whatman No. 1, England) to remove silica precipitates. The concentration of Co and Cr present in faecal samples was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), (Varian Vista Radial MPX Inductively Coupled Plasma – Optical Emission Spectrometer). Samples were decanted into 15 ml glass ICP test tubes and processed under standard conditions by reference to the regression curve created from working standards.

A two rumen pool model (Figure 1) with lags based on those described by Aharoni et al (1999) was fitted using WinSAAM (Stefanovski *et al*, 2003; See also http://www.winsaam.com/). The model was iterated until sums of squares was minimised.

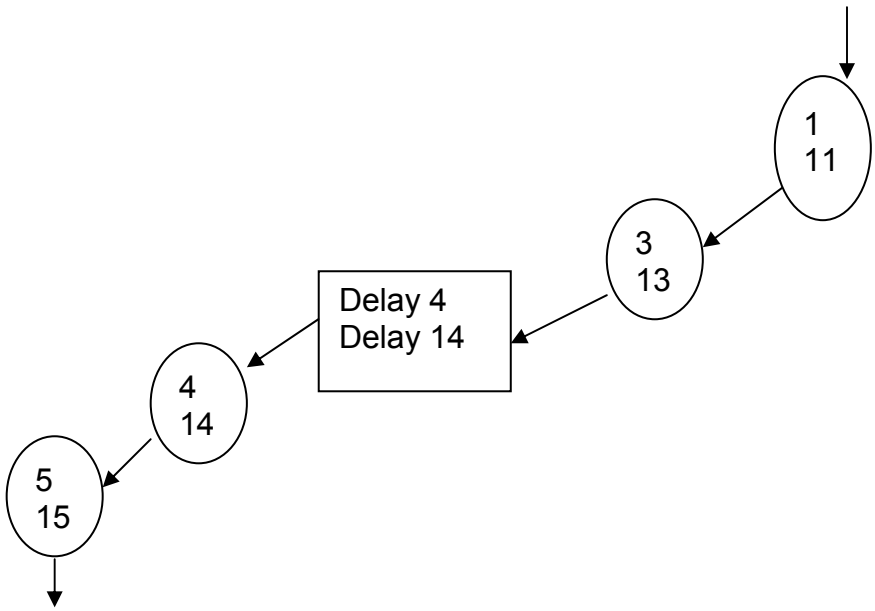

Figure 1. The model used to fit concentration of Cr (pools 1, 3, 4, 5 and delay 4) and Co (pools 11, 13, 14, 15 delay 14) in faeces following administration of a bolus does of Cr mordanted feed and Co-EDTA into the rumen. Model derived from Arahoni et al, 1999 to generate best fit to the observed concentration of Cr and Co marker in faeces.

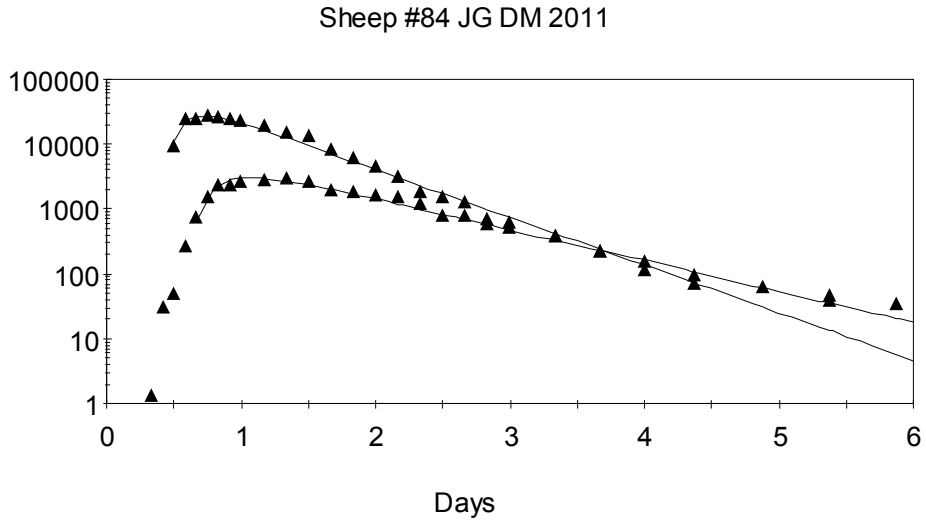

Figure 2. Example output from fitting the concentration of Cr (bottom line) and Co (top line) to the model described in Figure 1. Vertical axis = log concentration of marker (Cr or Co) in faeces DM (ug/g) and horizontal axis days.

Estimation of rumen volume and weight of contents using X-ray computer tomography
The volume and weight of the rumen was estimated by x-ray computer tomography (CT) which was modified from the methods described by Haynes *et al.* 2010 and 2011. Prior to scanning the ewes were withheld from morning feeding. The ewes were scanned live and without sedation using a Picker UltraZ 2000 CT scanner (Phillips Medical Imaging Australia, Sydney, New South Wales, Australia). The sheep were placed ventrally into a semi-cylindrical cradle, with their front and hind legs tucked in underneath their bellies, and restrained with by strapping the animals into the cradle and supported with foam pads.

Animals were pacified by placing a towel over their heads. The torso of each sheep was scanned were approximately 44 to 61 (depending on the size of the animal) serial 5 mm cross-sectional images taken at 15 mm interval. The torso region scanned was started at approximately the 3rd or 4th thoracic vertebrae and finishing at the 1st or 2nd caudal vertebrae.

To determine the volume and weight of the rumen (including both the atrium and ventral sac of the rumen) the CT images were individually edited to remove all other internal organs and digestive tract using the Apple open-source software program Osirix (Rosset *et al*. 2004). The weight of the rumen was the estimated using the software program Autocat (Thompson and Kinghorn 1992). The volume of the rumen was estimated by Osirix using a volume rendering algorithm provided. The volume rendering algorithm measured the area inside the region of interest in the rumens and computed the according rumen volume using the algorithm described by Rabtib *et al.* (2009):

$$Volume = \frac{8}{3\pi} \frac{Horizontal\ Area * Vertical\ Area}{Length}$$

Estimation of total purine excretion.

Statistical analysis Differences between high and low methane producing groups, for all characteristics of interest were determined using 2-tailed t-tests.

Results

Methane production per day per unit intake (CH_4/DMI), remained significantly different between high and low groups (P=0.005) on retesting (after their initial selection), but the groups did not differ in liveweight, DMI or apparent whole tract digestibility (Table 1). Lower CH_4/DMI was associated with less particulate matter (contents DM) in the rumen (P=0.007), fewer contents as a fraction of food eaten (P=0.002) and a shorter particle mean rumen retention time (P=0.002) (Table 2). These findings were corroborated by the results of computer tomography, indicating that animals producing less CH_4/DMI had smaller rumens and less rumen contents (particulate plus liquid) (Table 3).

Table 1. Summary of data (mean ± SEM) from ewes selected on basis of difference CH_4 g/d/ kg DMI). Data are from 10 "high" and 10 "low" emitters.

Group	High		Low		Significance
Liveweight (kg)	61.4±3.6		58.7±3.6		N.S.
Dry Matter Intake (kg)	1.03±0.06		0.99±.0.06		N.S.
Dry Matter Digestibility (%)	66.4±0.92		64.8±1.10		N.S.
CH_4 (g/kg DMI)	22.9±.032		20.74±0.56		P=0.005

Levels of significance derived from a 2 sample t test. NS = P>0.1

Table 2. Summary of data (mean ± SEM) from ewes selected on basis of difference CH_4 g/d/ kg DMI). Mean retention time and rumen contents/feed eaten were derived from study of faecal collection of animals dosed with feed mordanted with Cr (particles) and CO-EDTA (liquid phase) markers. Data were derived using WinSAAM to estimate pools sizes and

fluxes between pools using a 2 pool model simplified from that described by Aharoni *et al* (1999).

Group	High		Low		Significance
Rumen Contents (kg DM)	0.685±0.031		0.549±0.031		P=0.007
Mean Particle Retention Time (d)	1.34±0.043		1.11±0.045		P=0.002
Rumen contents/feed eaten.	0.669±0.021		0.557±0.023		P=0.002

Levels of significance derived from a 2 sample t test. NS = P>0.1

Table 3. Summary of data (mean ± SEM from ewes selected on basis of difference CH_4 g/d/ kg DMI). Weight of Rumen contents, Rumen volume, Gas phase/ volume were derived from CT scanning of the fed live animals at the completion of measurement DMP. Rumen DM% is derived from weight of particles (estimated using Cr mordanted feed and Co EDTA markers (Table 2) / weight of rumen contents calculated from CT scanner data.

Group	High		Low		Significance
Weight Rumen Contents (kg)	5.42±0.411		4.43±0.32		P=0.074
Rumen Volume (l)	7.42±0.56		5.91±0.44		P=0.048
Rumen Gas Space Proportion	0.267±0.015		0.247±0.018		N.S.
Rumen est. DM%.	12.96±0.59		12.78±0.91		N.S.

Levels of significance derived from a 2 sample t test. NS = P>0.1

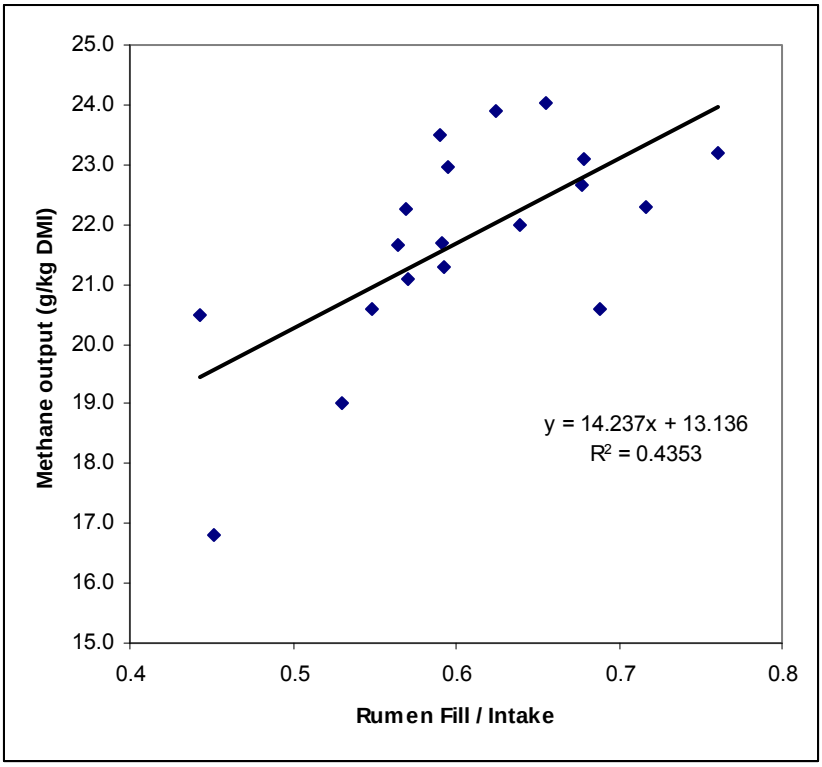

Figure 3. The relationship between CH_4 production (CH_4 g/ kg dry feed ingested) and the proportion of ingested feed retained in the rumen. Note this figure is almost identical with the relationship between CH_4 production (g/d/kg DMI) and mean retention time in the rumen CH_4 = 13.1±2.39 +7.19±1.96* Rumen MRT, R^2 = 44.1

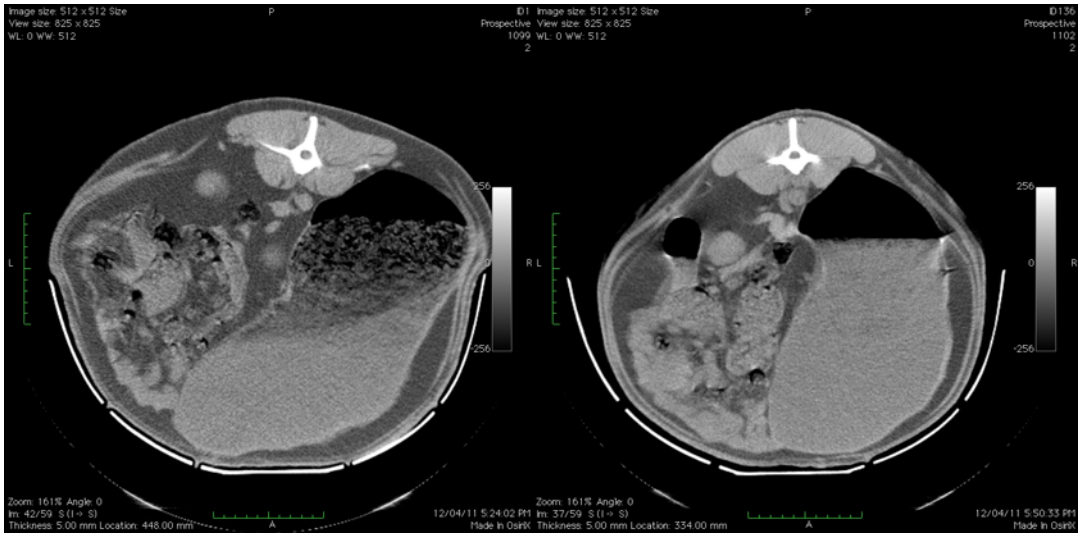

Figure 4: Example of rumen content morphology of High and Low methane producing sheep on fixed intake, after 12h fasting. Left – high methane producer (22.06g CH_4/ kg intake); Right - low methane producer (18.26g CH_4/ kg intake).

Discussion

The data indicate that methane production is affected by the time feed particles spend in the rumen (or the proportion of ingested feed retained in the rumen). The data suggests that high methane emitting sheep have larger rumens than sheep with low(er) emissions, but that the proportion of rumen (organ) volume as gas is similar and that the dry matter percentage of the rumens is similar between high and low emitters. This is consistent with the notion that rate of passage of feed through the rumen is one factor contributing to systematic variation in methane production. The data are also consistent with the idea that this is in part due to intrinsic differences in rumen volume.

Data exploration to date is incomplete, but provides promising possibilities for further elucidation of the observed differences. Further interrogation of existing data will lead to greater understanding of the influence of longer term (>1d) intake on methane production. Analysis of the liquid phase of rumen outflow, in conjunction with analysis of microbial protein outflow (not yet complete) is expected to provide insghts into the potential differences in efficiency in NAN absorbance between high and low emitters. There remain a number of avenues in exploring the results of CAT investigations. For example, empirically there appear to be significant difference in the morphology of rumen contents between high and low emitters. This avenue is at a preliminary stage, where we are attempting to develop a mathematical model and sampling protocol to deal with the data in an objective manner.

If these observations were repeated in progeny of animals selected for and against methane they would suggest that selection is for some morphological / functional arrangement rather than methane production / unit feed fermented. A potential consequence of this might be that low emitting animals (via this mechanism) would not be as productive on lower quality feeds.

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Appendix 7.

Feed-use efficiency in relation to methane emissions in growing Merino lambs[2]

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Abstract
This paper reports on four experiments that tested the hypothesis that improved feed use efficiency would be negatively related to methane production and that these traits would be associated with breeding values for muscling and fat. More than 900 Merino progeny between 5 and 14 months of age with Australian Sheep Breeding values for post-weaning liveweight (PWT), fat (PFAT) and muscle (PEMD) were fed ad libitum for 35 to 50 days and daily feed intake and liveweight gain were measured to estimate feed conversion ratio (FCE) and residual feed intake (RFI). Methane production was measured twice on each animal using portable chambers to estimate daily methane production. Across all experiments, the average feed intake, liveweight gain, feed use efficiency and methane production of progeny from different sires varied by 30 to 40%. The differences in daily methane production were still evident after adjustment for differences in liveweight of progeny. Sheep with higher PWT consistent grew faster, ate more and produced more methane per day. The effects of PFAT and PEMD were less consistent, with some evidence that higher PFAT and lower PEMD were associated with higher daily methane production. However, across all experiments, there was little evidence that feed use efficiency, either RFI or FCE, explained significant variation in daily methane production. Daily methane production was most closely related to average liveweight $r^2 = 0.26$; $P<0.001$) and liveweight change ($r^2 = 0.18$; $P<0.001$) during ad libitum feeding period, and only weakly related to average feed intake ($r^2 = 0.04$; $P<0.05$). While it appears that large differences exist in methane production between sire groups, is not obvious from the analysis undertaken to date how such animals could be selected without compromising performance.

Introduction
The feed conversion efficiency of a sheep flock focused on lamb production is defined as the amount of lamb produced in relation to the amount of feed eaten by the ewe and her progeny over a year. Whole flock efficiency can be increased by increasing net reproductive rates, reducing adult mortality rates, increasing growth rates of progeny without increasing ewe maintenance requirements and increasing feed use efficiency (Goddard *et al*. 2011). Residual feed intake is a measure of feed efficiency and is the difference between the amount of feed eaten by an animal and the amount of feed expected to have been eaten by the animal based on its size and level of production. Animals that eat less than expected based on their size and level of production have a lower (negative) RFI and are more efficient than those with a higher (positive) RFI. Residual feed intake is moderately heritable in beef cattle and those with a low RFI eat less but perform similarly to those with a high RFI (reviewed by Herd and Pitchford 2011). Feed-use efficiency in beef cattle has been assigned an Estimated Breeding Value (EBV) to quantify the genetic potential of the animal regarding its feed-use efficiency. There is some evidence that RFI is also heritable in Merino sheep (Allington *et al*. 2011), and other sheep breeds (Cammack *et al.* 2005), however it is

[2] This is the first draft of a paper has been prepared for a milestone report only. A more comprehensive analysis and interpretation of the data will be completed and the paper modified accordingly before submission.

extremely unlikely that breeding values for RFI will be adopted by the Australian sheep industry because of economic and practical constraints of measuring feed-use efficiency on large numbers of sheep. Therefore if the sheep industry is going to be able to improve feed-use efficiency there is a need to identify correlated traits that are relatively cheap and simple to measure.

The sheep industry in Australia utilizes Australian Sheep Breeding Values (ASBV's) to achieve genetic gain in Merino sheep (Brown *et al.* 2007). Finding a genetic correlation between an ASBV and feed-use efficiency would be the most appropriate indirect selection criteria used as ASBV's are already used in the industry. There are three traits that are important to select for in maternal sheep, including the depth of the *longisimus dorsi* muscle (PEMD), subcutaneous fat (PFAT) and weight (PWT) at post-weaning age (210 days old) and ASBVs exist for all these traits. Reduced feed-use efficiency is associated with higher fat percentage in cattle (Shaffer *et al.* 2011) and in pigs (Arthur *et al.* 2009). This is explained by the lower energy requirement of depositing lean tissue compared to the deposition of the same weight of fat (Herd and Arthur 2009). In beef cattle, animals selected for post weaning weight have no significant change in measures of feed-use efficiency indicating that growth has no effect on feed-use efficiency (Arthur *et al.* 2001). This suggests that selecting for low PFAT and high PEMD is likely to increase feed-use efficiency, and these traits may be useful indicator traits of feed-use efficiency in sheep.

Sheep produce methane as a by-product of fermentative digestion in the rumen and hind gut, and methane production accounts for about 19% of gross energy intake. Feed intake typically accounts for about 80% of the variation in daily methane production in grazing ruminants, however methane production may also be under the animals control as marked differences in methane production per kilogram of dry matter intake have been observed among animals consuming the same ration (Blaxter and Clapperton 1965, Pinares-Patino *et al.* 2003, Goopy *et al.* 2006). As selection for lower RFI is associated with reduced feed intake, selection for low RFI should also reduce methane emissions, nitrogen excretion and manure production (Herd *et al.* 2002; Arthur *et al*. 2010). Hegarty *et al.* (2007) reported that cattle selected for lower RFI emitted between 18 to 60% less methane per day than cattle selected for high RFI. It would appear that indirect selection for lower RFI could improve profitability and reduce methane emissions from sheep production systems.

This paper reports on four experiments undertaken to test the hypothesis that lambs with a high ASBV for PEMD will be more efficient than those with a low PEMD, and lambs with a high ASBV for PFAT will be less efficient than those with a low PFAT. We also hypothesized than lambs that were more efficient would produce less methane.

Materials and Methods

All procedures reported were conducted according to the guidelines of the Australian Code of Practice for the Use of Animals for Scientific Purposes and received approval from the Western Australian Department of Agriculture and Food Animal Ethics Committee.

Experiments and animals

Merino lambs with full pedigree information and ASBVs for growth, fat and muscling at post-weaning age were sourced from the Maternal Efficiency Flock at Pingelly (32^0 32'S, $117^0$05E) and the Sheep CRC Information Nucleus flock at Katanning. The animals used varied from 137 to 400 days old and 28.1 to 56.4 kg at the start of the feed efficiency measurements and from 177 to 436 days old and 37.9 to 63.9 kg at the time of the methane measurements. A summary animals used in each of the experiments is given in Table 1.

Table 1. Summary of sheep from Maternal Efficiency Flock (MEF) and Information Nucleus Flock (INF) used in Experiment 1, 2 and 3 to measure feed use efficiency and daily methane production.

Experiment and flock	Sex	Number of animals	Age at start of feed efficiency measurements (kg)	Liveweight at start of feed efficiency measurements (kg)	Age at start of methane measurements (days)	Liveweight at start of methane measurements (days)
Experiment 1						
- MEF (2009 born)	Rams	162	215	50.3	247	54.7
- MEF (2009 born)	Ewes	135	262	43.1	304	51.2
Experiment 2						
- INF (2009 born)	Wethers	76	400	56.4	436	63.9
- INF (2009 born)	Ewes	98	325	47.4	359	55.6
Experiment 2						
- MEF (2010a born)	Rams	194	200	41.5	223	49.0
- MEF (2010a born)	Ewes	191	147	35.1	184	41.8
Experiment 4						
- MEF (2010b born)	Rams	48	137	28.1	177	36.1
- INF (20010b born)	Ewes	41	187	36.2	229	46.2

The lambs were transferred from their flock of origin to a feedlot facility at the Medina Research Station about 10-days prior to commencement of feed intake and efficiency measurements. They were stocked at less than 100 lambs per feedlot (60 m x 20 m) and each feedlot was fitted with a water trough, self feeder and hay rack. During this 10-day introductory period the lambs were offered straw ad libitum plus increasing amounts of a commercial pellet (90% dry matter, 12.5 MJ metabolisable energy/kg dry matter and 16% crude protein) such that after 10-days the lambs were consuming pellets *ad libitum*. The lambs were then housed indoors for 40 to 50 days in up to 15 pens (3.3 x 7.5 m) at a maximum stocking density of 17 lambs per pen.

Measurements

Each of the 15 pens in the feed intake facility were fitted with a water trough and automated feeding units capable of weighing feed intake to the nearest 10 g. Sheep were identified by electronic tags and the feeding units were fitted with electronic tag readers that could identify individual sheep each time they were feeding. Only one sheep could be feeding from each unit at the same time. The feeders were also fitted with a load bar and scales to enable total feed intake and the number of meals for each individual sheep to be recorded each day. During the 40 to 50 day test period the lambs were weighed twice per week and at the end of the period all lambs had their depth of fat and muscle at the C-site measured by ultrasound.

Calculation of Residual Feed Intake and Feed Conversion Efficiency

The mean liveweight was modelled over time separately for each animal using a random coefficient regression including a cubic spline for time (Verbyla *et al*. 1999). The model fitted was:

Liveweight = m + day + animal + animal.day + spline(day) + animal.spline(day).

The term ‘day’ was fitted as a fixed effect while all other terms were fitted as random effects, with a covariance between the animal intercept (animal) and slope (animal.day). The likelihood ratio test was used to assess any spline effects after the previously mentioned terms (day, animal and animal.day) had been fitted. All statistical analyses were performed using GenStat (GenStat Committee 2008).

The average liveweight and the liveweight at the start and end of the test period predicted from the model above were used to calculate RFI and FCE. The total mount of pellets consumed by the lambs divided by their liveweight gain over the test period was used to derive FCE. Residual feed intake was calculated using the current Australian beef cattle model taken from Knott *et al.* (2008). The predicted average liveweight was raised to the power of 0.73 to calculate mean metabolic liveweight. Mean metabolic mid weight (MMWT) was included in the model as a predictor of maintenance requirements and average daily gain (ADG) was included as a measure of growth. The model fitted to calculate RFI was:

$$Yi = \beta 0 + \beta 1\text{ADG}i + \beta 2\text{MMWT}i + ei \quad (3)$$

where Yi = daily FI of animal i, $\beta 0$ = regression intercept, $\beta 1$ = partial regression coefficient of feed intake on ADG, $\beta 2$ = partial regression coefficient of FI on MMWT, and ei = residual error in FI of animal i. The residual portion was predicted for each animal, this is the RFI..

Measurement of methane emissions

Methane production from individual sheep was measured using 16 portable methane chambers (122cm x 122cm x 56cm) set up in an insulated animal house adjacent to the feed intake facility. Methane production for each sheep was measured twice during the last 1 to 3 weeks of the 35 to 50 day period of *ad libitum* feeding. The portable chambers were constructed to trap all exhaled and eructed gases during a one hour collection period and the development and full description of the chambers is provided by Goopy *et al*. (2011). Individual sheep were removed from their pens used to measure feed intake into a race and to a position below an individual chamber which was suspended above the raceway. The chamber was then lowered over the sheep and the chamber secured within with in 15 minutes of being removed from access to feed. A thermometer was mounted in each chamber and temperature was recorded for each animals at start and end of measurement

period. Methane concentration is the chambers was measured 20, 40 and 60 minutes after the chamber was secured using a MicroFID flame ionization detector fitted with a 20 cm flexible silicon sampling tube which was introduced to each chamber through a sampling port. After the final measurement sheep were returned to their pens in the feed intake facility. Total gas space inside the portable chamber (*i.e.*, net volume) was estimated by assuming that the volume occupied by the sheep was equal to 1 L/kg liveweight and subtracting the liveweight of the sheep from the internal volume of the chamber. Methane production during the period was estimated as methane concentration corrected for background methane concentration multiplied by net chamber volume. The estimated production in the chamber was then converted to estimated production over 24 hours.

Statistical Analysis

The method of restricted maximum likelihood (REML) was used to fit average intake, liveweight gain, RFI, FCE and daily methane production with ASBV for growth (PWT), fat (PFAT) and muscle (PEMD) and sex as fixed effects and sire effects random. For methane produced per day REML) was used to model RFI and sex as fixed effects and pen, run, box and sire effects as random. All statistical analyses were performed using GenStat (GenStat Committee 2008) but all analysis reported in this draft paper are preliminary only. For all analyses first order interactions were included in the starting model, and removed in a stepwise process if non-significant (P>0.1).

Results

Live weights and feed intake

Liveweight of sheep during the ad libitum feeding periods in each experiment are shown in Figure 1. Average growth rates were 221 g/day, 247 g/day, 246 g/day and 287 g/day in Experiments 1 to 4 respectively. On average, male lambs grew about 8% faster than female lambs (255 vs. 235 g/day; P<0.05) but there was no significant difference in growth rates between single and twin born lambs (248 vs. 241 g/day; P>0.05). In all experiments there were significant differences (P<0.001) in the average liveweight gain of progeny from different sires; Experiment 1 (218 to 276 g/day); Experiment 2 (181 to 259 g/day); Experiment 3 (215 to 264 g/day) and Experiment 4 (233 to 311 g/day).

Average intake of feed per day for the different experiments was 1.75 kg/day, 1.62g/day, 1.58 kg/day and 1.48 kg/day in Experiments 1 to 4 respectively. On average, male lambs ate about 5% more than female lambs (1.66 vs. 1.57 kg/day; P<0.05) and single lambs ate about 4% more than twin born lambs (1.64 vs. 1.57 kg/day; P<0.05). In all experiments there were significant differences (P<0.001) in the average of intake per day by progeny from different sires; Experiment 1 (1.59 to 2.01 kg/day); Experiment 2 (1.56 to 2.36 kg/day); Experiment 3 (1.64 to 2.02 kg/day) and Experiment 4 (1.38 to 1.79 kg/day).

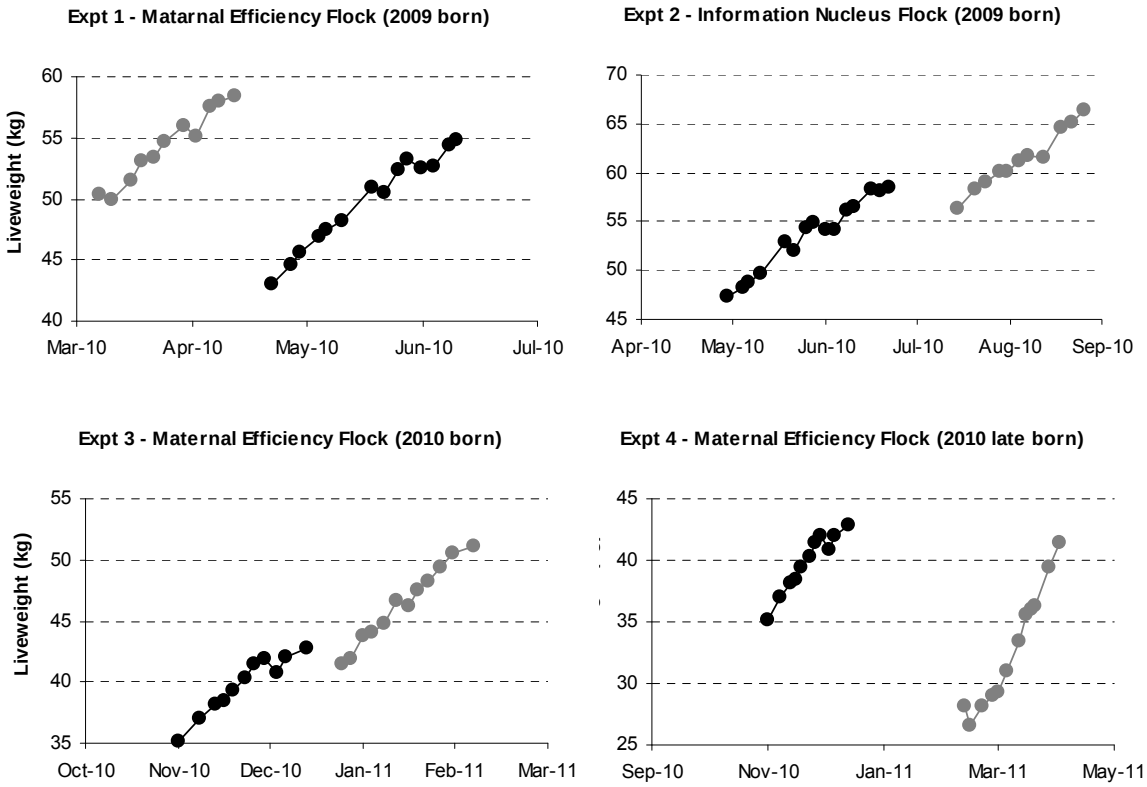

Figure 1. Liveweights of Merino rams (grey) or ewe (black) lambs housed indoors and fed *ad libitum* high quality pellets over 35 to 50 day periods in each of four experiments.

Feed use efficiency

There was a positive correlation between the FCE and the RFI of the lambs in all experiments (Figure 2). The average FCE for the different experiments was 7.6, 9.0, 7.6 and 6.1 kg DM consumed/kg liveweight gain in Experiments 1 to 4 respectively. On average across all experiments, there was no significant difference in feed use efficiency between the male and female lambs (FCE, 7.6 vs. 7.8; RFI, -0.011 vs -0.004) but single lambs were slightly less efficient than twin born lambs (FCE, 7.8 vs. 7.5; RFI, -0.0002 vs. -0.033). In all experiments there were significant differences ($P<0.001$) in the FCE and RFI of progeny from different sires; Experiment 1 (6.6 to 8.6 and -0.37 to +0.32); Experiment 2 (7.3 to 11.1 and -0.48 to 0.96); Experiment 3 (6.3 to 9.9 and -0.31 to 0.55) and Experiment 4 (5.2 to 7.1 and -0.55 to 0.49).

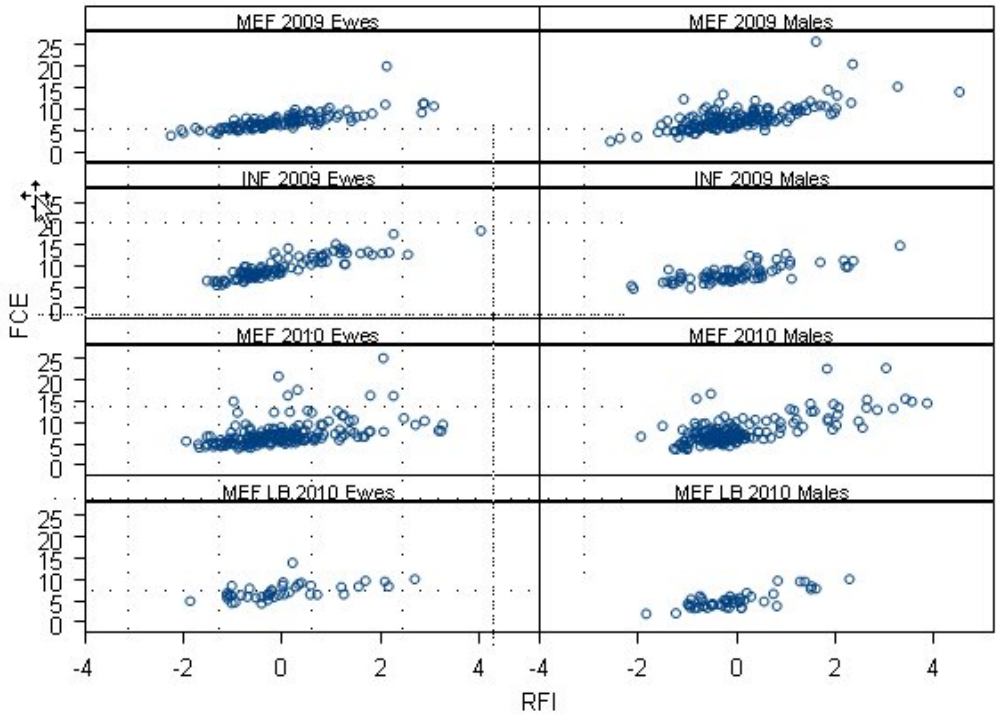

Figure 2. Relationship between residual feed intake (RFI) and feed conversion efficiency (FCE) for progeny in four experiments; Experiment 1, females (r^2 = 0.60; P<0.001) and males (r^2 = 0.46; P<0.001); Experiment 2, females (r^2 = 0.79; P<0.001) and males r^2 = 0.55; P<0.001 (); Experiment 3, females (r^2 = 0.20; P<0.001) and males (r^2 = 0.49; P<0.001); and Experiment 4, females (r^2 = 0.32; P<0.001) and males (r^2 = 0.67; P<0.001).

Daily methane production

Methane production was measured twice on each animals and the average repeatability between measurements was about 0.40. Average methane production per day for the different experiments was estimated to be 55 L/day, 61 L/day, 68L/day and 55 L/day in Experiments 1 to 4 respectively. On average across all experiments, male lambs produced about 10% more than female lambs (65 vs. 59L/day; P<0.05) and single lambs similarly produced about 10% more than twin born lambs (64 vs. 59L/day; P<0.05). These differences between the sex and birth type of the lambs were largely explained by differences in the liveweights. In all experiments there were significant differences in the average methane production from progeny from different sires; Experiment 1 (50 to 69 L/day; P<0.1); Experiment 2 (46 to 76 L/day: P<0.05); Experiment 3 (60 to 77 L/day: P<0.05) and Experiment 4 (47 to 62 L/day: P<0.05). In all cases these differences between sires persisted and were of similar magnitude after adjustment for differences in liveweight of progeny.

Intake, growth and feed use efficiency in relation to ASBVs for growth, fat and muscle and methane production

As expected, there was a consistent positive relationship between ASBV for PWT and average daily gain in all experiments (P<0.05); one unit increase in PWT was associated with an extra 7 to 22 g/day liveweight gain during the *ad libitum* feeding period. Lambs with higher ASBVs for PWT also had significantly higher daily feed intakes in all experiments (0.26 to 0.59 g/day per one unit increase in PWT). As a consequence, PWT was not associated with either FCE or RFI but PWT was significantly (P<0.001) related to daily methane production in all experiments (average +2.7 L/day of methane per unit PWT).

There was generally no significant effects of ASBV for PFAT or PEMD on liveweight gain. However, in two of the four experiments, there was a tendency for higher PFAT to be associated with increased daily feed intakes and increased RFI (reduced feed use efficiency) and daily methane production. Similarly, in one of the four experiments higher PEMD was associated with significantly lower daily feed intake (P<0.001), reduced RFI (higher feed use efficiency, P<0.05) and reduced daily methane production (P<0.01).

Across all experiments, there was no significant relationship between RFI and daily

methane production (Figure 3). For two of eight groups of sheep FCE was negatively correlated with daily methane production but only explained 4 and 9% of the variation in daily methane production. Daily methane production was most closely related to average liveweight r^2 = 0.26; P<0.001) and liveweight change (r^2 = 0.18; P<0.001) during ad libitum feeding period, and only weakly related to average feed intake (r^2 = 0.04; P<0.05).

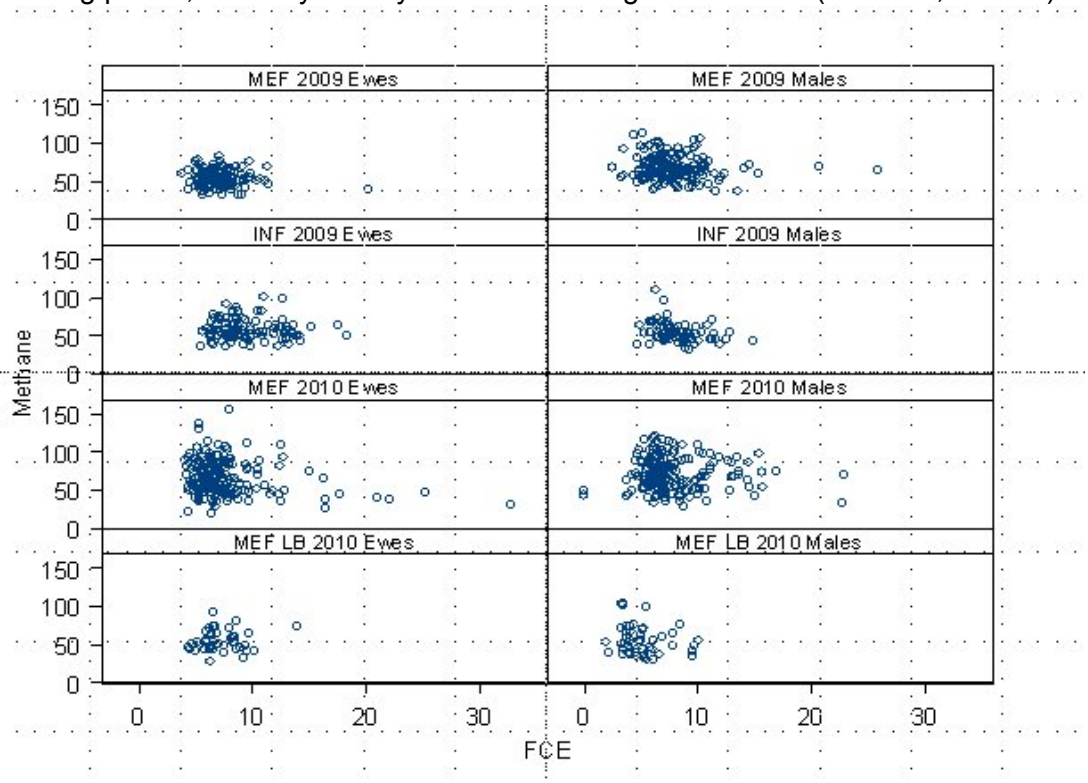

Figure 3. Relationship between feed conversion efficiency (FCE; kg dry matter intake/kg liveweight gain) and daily methane production (L/day) for progeny in four experiments.

Discussion

There was large variation in the average feed intake, liveweight gain, feed-use efficiency and methane production of progeny from different sires. Feed intake and liveweight gain are both heritable traits and other analysis of the data reported in this paper indicated that the feed-use efficiency of growing Merino lambs was related to that of their dams measured at a similar age and under similar conditions (Allington *et al.* 2011). This suggests that these measures of feed-use efficiency are under genetic control, which is consisted with studies using ram lambs (Snowder and Van Vleck 2003; Cammack *et al.* 2005), cattle (Arthur *et al.* 2004), pigs (de Haer *et al.* 1993) and chickens (Luiting *et al.* 1991). Together with the observed variation in both FCE and RFI, these results suggest that there is scope to select for feed-use efficiency in Merino lambs. However, to the economic and practical constraints of measuring feed-use efficiency on large numbers of sheep, if the sheep industry is going to be able to improve feed-use efficiency there is a need to identify correlated traits that are relatively cheap and simple to measure.

There is some evidence in this study that ASBVs for growth, fat or muscle could be related to feed use efficiency and its components. As expected, there was a consistent positive relationship between ASBV for PWT and average daily gain in all experiments. Lambs with higher ASBVs for PWT also had significantly higher daily feed intakes in all experiments such that PWT was not associated with either FCE or RFI. . This is consistent with other studies in beef cattle, where it has been shown that there is large variation in feed-use efficiency between individuals, independent of growth rates or post-weaning weight (Arthur *et al.* 1997). In some of our experiments there was a negative relationship between PFAT and feed use efficiency as lambs with a higher PFAT ate more but this was not reflected in higher growth rates. There is considerable evidence that suggests that cattle (Fox *et al.* 2004; Shaffer *et al.* 2011; Herd and Arthur 2004) and pigs (Arthur *et al.* 2009) with a high fat percentage will be less efficient at converting feed into liveweight gain due to the relative expense of depositing fat compared to lean (Herd and Arthur 2009). In contrast to PFAT, we found some evidence that improved efficiency was associated with higher PEMD due to relatively lower intakes compared to liveweight gain. This is again consistent

with results indicating that cattle with higher muscle are more efficient (Herd and Arthur 2009). Further work is in progress to confirm these relationships between ASBVs for fat and muscle in sheep and their feed use efficiency. In cattle associations between single nucleotide polymorphism and RFI are promising with SNP panels accounting for 37% of variation in RFI (Sherman *et al*. 2010), suggesting that similar work in sheep is warranted.

Sheep with higher PWT consistently grew faster, ate more and produced more methane per day; on average, a one unit change in PWT was associated with an extra 2.7L methane/day or an increase of about 5%. However there was little evidence in this study with over 900 animals that feed use efficiency, either RFI or FCE, explained significant variation in daily methane production. Of all the parameters measured daily methane production was most closely related to average liveweight (r^2 = 0.26; P<0.001) and liveweight change (r^2 = 0.18; P<0.001) during ad libitum feeding period, but only weakly related to average feed intake (r^2 = 0.04; P<0.05). As feed intake was the major driver of differences in FCE and RFI, and RFI is also independent of liveweight and liveweight change, it is not surprising that feed use efficiency was not well related to methane production. Further work is required to quantify the effects of feed intake immediately prior to methane measurements on methane production and undertake a combined analysis to quantify the phenotypic and genetic associations between various traits and both total methane production and methane production per unit of dry matter intake.

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