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## Genetics to reduce methane emissions from Australian sheep

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# Executive summary

Methane emissions by sheep are heritable. There are no strong (positive or negative) relationships between production traits (other than feed intake) and methane emissions. It is possible to use methane emissions in breeding objectives to reduce feed costs while at the same time limiting methane emissions even in the absence of a price on carbon. If there is a price on carbon, including methane measurements in a breeding objective maintains profit and further reduces methane emissions. The trait that best suits a practical breeding objective is methane production (adjusted for weight), rather than methane yield (methane production divided by feed intake). Measurements of total methane production (adjusted for weight) using portable chambers have a high genetic correlation with measurements made in respiration chambers if the animals are eating the same feed. Essentially, this means that portable chambers can provide reliable data on methane emissions for the purposes of genetic selection. The best time (stage of life) to make methane measurements for genetic improvement is when the animals are dry (non-pregnant, non-lactating). Major genes affecting methane production are unlikely to be present, but use of genomic breeding values is possible.

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# 1. Background

In Australia, animal agriculture produces 60-70% of agricultural GHG. Most of the emissions from animal agriculture are from enteric methane ($CH_4$, derived from digestion of feed). Current estimates indicate that enteric methane accounts for approximately 9% of Australia's total GHG emissions. Reducing enteric methane emissions is an emerging issue for agriculture in many countries. Of the possible options to mitigate methane emissions from livestock, breeding animals that have lower methane emissions is considered to be the one most likely to provide sustainable low cost mitigation in extensive grazing situations (Buddle *et al*. 2011; Pickering *et al*. 2013).

To implement a selective breeding strategy to reduce methane emissions requires that the trait is heritable, preferably not associated with detrimental animal production outcomes, and capable of being measured on many animals at, preferably, low cost. It is now clear that methane emissions, and yield of methane per unit of feed eaten are heritable traits (Pinares-Patino et al. 2013; Donoghue et al. 2015, summarised by Pickering et al. 2015). Methods of measurement that are not costly have been established for sheep (Goopy et al. 2011), but using these methods for measurement of methane emissions by grazing animals for genetic evaluation could benefit from additional technical evaluation.

The key research questions were:

a) What is the best measurement protocol for managing animals prior to and during data collection, and the number of measurements required including timing of repeat measures?

b) What is the best time to measure an animal - this is essentially a question of whether, and to what extent, measures taken at different times in an animal's life are correlated?

c) What is the appropriate methane trait for genetic selection

d) What are the genetic parameters for the appropriate trait and correlations with production traits

This project addresses these research questions. It had two phases. The first was to address the research questions above and develop a robust protocol for measurement of methane emissions by grazing sheep. The second was to employ the best bet protocol to measure 2000 sheep from industry resource flocks on which other production traits have been measured and estimate genetic parameters for methane traits and the genetic relationship between methane and production traits. Information collected on methane production across a breeding cycle obtained in pursuit of b) can also inform the GHG accounting process, which at present assumes a single relationship between feed eaten and methane emissions across sheep of all physiological states and ages.

The aim of this project was to establish a reliable and cost effective procedure for measuring methane emissions from sheep, to measure sufficient sheep to estimate genetic parameters (heritability and genetic and phenotypic correlations between methane and production traits), to establish phenotypic and genomic breeding values and to discuss these with the sheep industry (Sheep Genetics). This information will enable ram breeders to breed sheep that produce less methane and to participate in the Emission Reduction Fund offset program.

# 2. Methodology

A diagram illustrating the interdependencies of activities in this project is shown in Figure 1. Overall co-ordination (Activity 1) of the project was administered through UNE and the Rumen Pangenome Project (RPP). These activities are not reported on here.

Subsequent project Activities (2-4) provided measurements, data, insights and samples for this project and the "Host control of methane emissions by sheep" project.

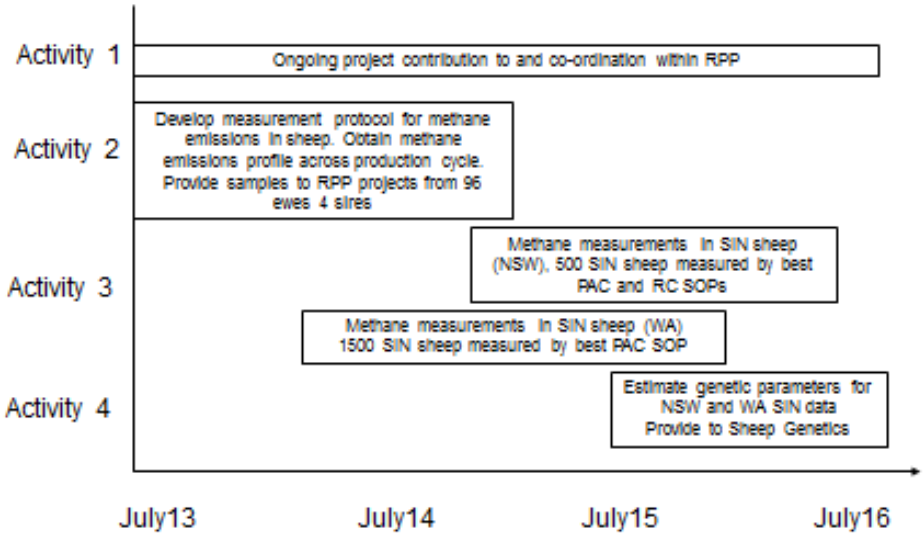


**Figure 1. Schematic of project activities, showing interdependence of activities and linkages to other RPP projects.**

## 2.1 Experimental data

### (a) Activity 2 - NSW

The development of the protocol to measure methane emissions was based on a data set collected from 96 ewes, approximately 12 months old from 4 sires (19 to 29 progeny per sire). The sires were identified as having divergent methane emissions in a prior study were measured for a wide range of traits. The ewes were transported from Glen Innes Research Station to UNE in October 2012, where they were housed in individual pens and offered a diet of chaffed lucerne and oaten hay (dry matter digestibility 65%, crude protein 14% DM) at 20% more than daily feed intake. Feed refusals were recorded each day. Water was available at all times. The ewes were weighed at 2 weekly intervals.

After 3 weeks adaptation to the diet methane emissions were measured on 2 occasions using Portable accumulation chambers (PACs, Goopy et al, 2011). Methane concentration was recorded 30 and 60 minutes after entering the chambers. Methane ($CH_4$) was measured using an FID analyser (MX100053 ENVCO Wellington New Zealand). Carbon dioxide ($CO_2$) concentration was recorded after 60 minutes. On the second (and subsequent) occasion(s), oxygen concentration was also measured after 60 minutes. Carbon Dioxide and oxygen ($O_2$) concentration was measured using a FoxBox (Sable Instruments, Nevada, USA). Feed was available up to the time ewes entered the chambers. Forty eight ewes were measured each

day over a period of 4 days. Feed intake on the day of measurement, and from 4pm the evening before and from 8 am to 4 pm the previous day was recorded. All prior measurements of feed intake were for 24 hour periods (8 am until 8 am). A week after the PAC measurements measurement of methane in respiration chambers (RC) commenced. Eight ewes were measured for 22 hrs each day. Intake was recorded prior to entering the chambers and in the chambers. Chambers were operated and instrumented as described by Bird *et al.* (2008). At the completion of the respiration chamber measurements, we conducted CT scanning on each ewe to enable visualisation and characterisation of the reticulo-rumen complex. Faecal and rumen fluid samples were collected.

The diet was the then altered to a maintenance level (calculated according to SCA, 1990) and after 2 weeks the PAC measurements were repeated. The ewes were computer tomography (CT) scanned and faecal and rumen samples collected while on a maintenance level of feed intake. The ewes were returned to Glen Innes Research Station at the end of this part of the study (mid December 2012).

In March 2013, field chamber measurements of methane, $CO_2$, $O_2$ and live weight were repeated while the ewes were grazing at pasture. The ewes were measured on 4 occasions, twice on each pasture type. Ewes were removed from pasture 60 minutes before being placed in the PACs.

In April 2013 the oestrus cycles of the ewes was synchronised and the ewes were joined in early May 2013. The ewes then returned to UNE where again measured in PACs and RC while eating 1.6* maintenance and in PACs while eating ad-lib. At the time of measurement they were between 3.5 and 4 months post-conception. The ewes were CT scanned and had rumen samples taken for microbial community evaluation and VFA analysis.

In July 2013, 96 of the above ewes were again housed in individual pens and offered a mix of chaffed lucerne and oaten hay at 1.6 times calculated maintenance requirement, irrespective of pregnancy status. Feed intake was recorded daily. Between 23 and 26 July the ewes were placed in PACs (two times) for an hour each time. Methane, $CO_2$ and $O_2$ concentrations were measured and rate of production / consumption of gases calculated. From 29 July 8 sheep / day were placed in respiration chambers for 22 hours where methane and $CO_2$ emissions and feed intake were recorded. The ewes were CT scanned on August 13 and 14 and weighed on August 16. Intake was changed to ad-libitum, and intake recorded each day from August 16. Each ewe was placed in a PAC for 1 hour twice between 20 and 23 August and Methane, $CO_2$ and $O_2$ measured. Samples of rumen contents were obtained and the ewes were weighed and returned to Glen Innes.

The ewes lambed in late September 2013. During November 2013 the ewes were measured for $CH_4$, $CO_2$ and $O_2$ using PACs while they were at pasture at Glen Innes Research Station. During the periods 5 to 8 November and 19 to 22 November 2013, while they had lambs at foot (and were lactating) the ewes were measured for $CH_4$, $CO_2$, $O_2$ over 40 mins in PACs (2 x each period), liveweight was recorded and samples of rumen contents taken for VFA and Microbial analysis.

The lambs were weaned in January 2014. Ewes returned to the animal house facilities at UNE Armidale in two batches of 48 from February to April 2014. They were fed 50/50 Lucerne:Oaten Chaff at, (in order), 1.5* Maintenance, Maintenance, ad-libitum (batch 1) and 1.5* Maintenance, ad-libitum and Maintenance (batch 2) Measurements of $CH_4$, $CO_2$, $O_2$

were made twice at each feeding level over 40 mins in PACs and they were measured in respiration chambers when offered feed at 1.5*Maintenance. Liveweight was recorded and samples of rumen contents taken for volatile fatty acid (VFA) and microbial analysis while they were on a maintenance ration. The ewes were CT scanned while eating a 1.5* Maintenance ration.

They were once again returned to Glen Innes Research Station, where they grazed pasture. Gas exchange was measured using PACs in the week commencing May 5. An illustration of the measurements and indicative feed intake eaten by the measured ewes is shown in Figure 2.

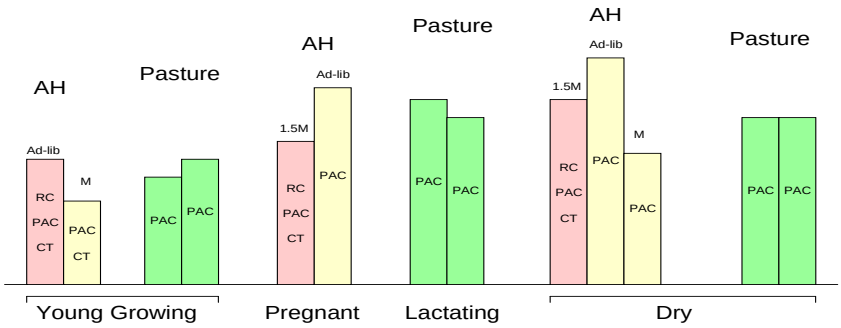


**Figure 2. Schematic of the experimental periods (physiological states – young growing, pregnant, lactating and dry – ewes after weaning of lambs), indicative levels of intake within periods (ad-lib, maintenance M, 1.5*M) and place of measurement (AH = animal house (UNE Armidale), pasture (Glen Innes Research Station) and some of the measurements made (RC = respiration chamber, PAC = portable accumulation chamber, CT = Computed tomography (X-Ray) scan) described in the results below. Height of bars indicatives estimated mean daily feed intake.**

### (b) Activity 3 - NSW

We sourced 508 ewes from the Sheep Information Nucleus Flock (INF) managed at UNE by the Sheep CRC. The majority of ewes were selected from 2012 and 2013 cohorts. However, we were able to source approx. 70 older ewes (2007 – 2009 drop) which had been previously measured for $CH_4$ adjusted for weight (Robinson et al, 2014). Each of these animals was genotyped with at least 12,000 SNP.

The measurement procedure was to bring animals into the Animal House in groups of 80 and offered feed (50/50 lucerne and cereal chaff) at 0800 each day at 1.5* maintenance requirement calculated on liveweight (water was available at all times). They were acclimatised to the animal house and feed regime for 1 week, then measured in PACs for 40 mins (as described above and later referred to as PAC0) separated by at least 10 days and in respiration chambers (as above) separated by at least 10 days and again in PACs for 40 mins after removal of feed 1 hr before measurement (PAC1). Rumen samples were obtained for

VFA and microbial composition analysis immediately after the second measurement in PACS. A blood sample was obtained from each ewe immediately prior to measurement in PAC for measurement of plasma acetate concentration.

Ewes (n=508) from the INF were used. They ranged in age from 1-8 years, and were from 184 sires. On average there were 2.8 progeny / sire (range 1-9). The ewes were brought indoors and housed in individual pens with access to feed at 1.5 * Maintenance requirement (based on liveweight) fed at 0800 daily and water (available at all times). The feed was a mix of equal parts of chaffed Lucerne and Cereal hay (Manuka Feeds, Quirindi, NSW). Chemical and estimated nutritive content is shown in Table 1.

**Table 1. Composition of feed used in NSW study. Values shown are mean (%) of 7 batches (+/- SD) Dry matter, Neutral Detergent Fibre (NDF) Acid Detergent Fibre (ADF) Crude Protein (N*6.25, CP), Ash, Organic Matter (OM) Estimated Digestible Dry Matter (DMD) Estimated Digestible Organic Matter (DOMD) and metabolisable Energy (ME, MJ/kg DM).**

| Dry Matter | NDF | ADF | WSC | CP | Ash | OM | DMD | DOMD | ME |
|---|---|---|---|---|---|---|---|---|---|
| 89.41 | 52.14 | 32.29 | 15.47 | 13.99 | 8.57 | 91.43 | 65.14 | 61.86 | 9.59 |
| 0.87 | 2.19 | 1.60 | 1.34 | 1.75 | 1.13 | 1.13 | 1.77 | 1.46 | 0.31 |

There were 7 measurement periods between May 2015 and April 2016. Three methods of measuring $CH_4$ production were used. Two utilised PACs, 1 the ewes were measured directly off feed in 4 batches of 12, 2 the ewes were measured 1 hr after feed was removed (2-3 batches of 12/day). The other used respiration chambers as described previously (Bird et al, 2008). Measurements in respiration chambers were for 22 hrs and were repeated (2 records / ewe) at an interval of at least 10 days. Measurements in PACs were for 40 minutes and some animals were repeated within and across days. In addition to measurement of $CH_4$ flux, $CO_2$ flux, and in PACs $O_2$ flux were measured as described above A jugular blood sample (5ml) was obtained prior to measurement using the 1 hour off feed PAC1 protocol and a rumen sample was obtained by stomach tube after the 1 hour of feed PAC1 measurement. A summary of measurements made in the NSW component of this work is shown in Table 2.

**Table 2. Summary of measurements made in NSW between May 2015 and April 2016. n = 508 animals. Note $CH_4/CO_2 = CH_4/CO_2*100$.**

| | Mean | Min | Max | SD |
|---|---|---|---|---|
| LWT | 50.22 | 34.40 | 74.50 | 7.04 |
| PAC0 (n=564) | | | | |
| $CH_4$ | 37.31 | 6.24 | 75.31 | 9.26 |
| $CO_2$adj | 449.30 | 231.30 | 734.90 | 84.00 |
| O2 | -468.00 | -731.40 | -267.20 | 76.51 |
| $CH_4/CO_2$ | 6.90 | 1.49 | 9.86 | 1.12 |
| MY | 44.15 | 19.44 | 101.62 | 9.85 |
| PAC1 (n=609) | | | | |
| $CH_4$ | 35.31 | 4.97 | 70.75 | 9.33 |
| $CO_2$adj | 397.50 | 207.40 | 704.60 | 72.93 |
| O2 | -436.40 | -732.50 | -257.80 | 75.22 |
| $CH_4/CO_2$ | 7.53 | 1.70 | 11.54 | 1.23 |
| MY | 40.03 | 4.78 | 72.88 | 7.20 |
| RC (n=1064) | | | | |
| $CH_4$ | 24.71 | 9.11 | 37.38 | 4.18 |
| CO2adj | 313.40 | 103.2 | 434.70 | 39.92 |
| $CH_4/CO_2$ | 7.513 | 4.39 | 20.30 | 0.77 |
| MY | 21.26 | 13.96 | 35.72 | 2.32 |

### (c) Activity 3 - WA study

The total data set included 1538 Merino and Maternal ewes from the INF. Testing for methane traits was conducted over three periods, the first in early summer 2014 (INF Follower lambs), the second in autumn 2015 (INF Follower ewes – Autumn) and the third in spring 2015 (INF Follower ewes – Spring). Only Merino ewes were used in the analysis. Therefore, 153 maternal ewes were removed and the final data set included records for 1,385 ewes. Each group is described in the further detail below. In the autumn group, 620

INF follower ewes were measured using PAC. The 189 sires of the INF follower ewes originated from 123 studs, including some non-Merino sires. Ewes were excluded from the analysis if sires could be identified as being non-Merino. The INF follower ewes in this group were born between 2007 – 2012 with lambing time ranging from late June to early August and weaning between September and November, depending on the year. The data set for analysis contains 464 animals and 923 records.

Methane production and production of $CO_2$ and $O_2$ were recorded on all ewes using PAC during February and March 2015 over four days each month. The ewes were measured directly off pasture on at least two occasions at least one month apart. A few sheep were measured between 3 – 5 times. At 6 am, prior to measurement, ewes were weighed and then drafted into two groups, one for measurement in the morning and the other in the afternoon. The ewes to measure in the afternoon were returned to pasture until lunchtime. A maximum of nine sessions per day were conducted with a maximum of 24 ewes per chamber and run. Total measurement time in the PACs ranged between 33 – 64 minutes. Initially measurement periods were 60 minutes in length, but it was established that gas measurements stabilise earlier and therefore the measurement period was reduced to 40 minutes. During each 40-60 minute PAC measurement gas measures were taken up to four times. Table 3 summarises the data on $CH_4$, $CO_2$ and the ratio of $CH_4/CO_2$ from PAC measurements, including live weight at time of measurement. In spring a second group of 593 INF Follower ewes was measured for methane traits using PACs. Forty-two of these ewes were also tested the previous autumn. Ewes in this group were born between 2007 and 2013. Ewes were measured over three days in late September and again over three days in late October 2015. Each day 7 - 8 runs were conducted with mostly 24 animals per run, but sometimes with only 20 – 23 per run. Two animals were identified as outliers and removed. Liveweight and gas production during PAC measurement are described in Table 3. The data sets contain 1253 records. INF follower lambs are the progeny of the INF follower ewes described in the previous two groups. All INF follower lambs were born from the beginning of April to the beginning of June 2014 and weaned mid-October. Three hundred and sixty eight female lambs were measured using the same protocol as previously described for the INF follower ewes. Measurements were conducted during three days at the end of November and 3 days at the end of December 2014. A maximum of eight sessions per day were conducted with a maximum of 20 lambs per run. During the period of each PAC measurement event 4 gas measurements were taken. Gas production during PAC measurement are shown in Table 3, including live weight at the time of measurement.

**Table 3. Descriptive statistics of measurements made in PACs (CH4, CO2 & O2, ml per min) and liveweight on the INF Follower ewes (autumns and spring) and INF Follower lambs at Katanning, WA.**

| | Follower ewes (autumn n=464) | | | Follower ewes (spring n=594 ) | | | Follower lambs (summer n = 366) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean (std dev) | Min | Max | Mean (±std dev) | Min | Max | Mean (±std dev) | Min | Max |
| $CH_4$ | 14.1 (4.8) | 1.4 | 44.1 | 15.8 (5.2) | 2.9 | 35.1 | 13.2 (5.3) | 0.1 | 37.8 |
| $O_2$ | -303.1 (68.5) | -566.1 | -140.6 | -445.3 (79.2) | -728.8 | -220.3 | -308.9 (56.9) | -590.3 | -135.5 |
| $CO_2$ | 382.7 (55.1) | 193.0 | 561.6 | 480.1 (89.5) | 221.2 | 890.7 | 300.7 (60.0) | 140.3 | 641.1 |
| $CH_4/CO_2$ *100 | 3.6 (1.0) | 0.4 | 13.3 | 3.27 (0.8) | 0.7 | 6.29 | 4.5 (1.8) | 0.02 | 9.8 |
| Live weight | 59.4 (7.1) | 36.0 | 83.0 | 58.5 (8.9) | 39.0 | 90.2 | 34.9 (5.6) | 17.6 | 66.5 |

### (d) Activity 4. Production traits (WA and NSW information nucleus sheep)

Production data was available on INF ewes from NSW and WA. Production data included live ultrasound scanning at the C-site (fat depth -CFAT) and eye muscle area (EMA)), wool traits (greasy fleece weight (GFW), fibre diameter (FD) and staple strength (SS)) and also weight traits at different ages (early post weaning weight (EPWT), post weaning weight (PWWT), yearling weight (YWT) and hogget weight (HWT). All production traits are described in Table 4. The number of records within each of the NSW and WA flocks are not ideal for the estimation of genetic correlations because in different years, different traits were recorded. Table 5 shows the number of records per stage (EPW, PW,Y, H) that were included in the analysis. Considering the low number of records, production traits of the WA and NSW flock were pooled and appropriate adjustments made in the model for analysis. It was demonstrated in a previous analysis that PAC1 $CH_4$ measured in NSW and WA is genetically the same trait. Consequently, also PAC1 $CH_4$ data was pooled across flocks.

**Table 4. Descriptive statistics (number of records (n), mean, minimum (Min), maximum (Max) and standard deviation (stddev)) of methane and production data recorded in NSW and WA on INF ewes.**

| | n | Mean | Min | Max | SD |
|---|---|---|---|---|---|
| NSW | | | | | |
| EPWT | 335 | 22.18 | 10.40 | 41.50 | 5.78 |
| PWWT | 506 | 29.50 | 16.20 | 45.40 | 5.28 |
| YWT | 509 | 35.29 | 17.00 | 59.00 | 6.57 |
| HWT | 395 | 39.81 | 22.20 | 61.00 | 6.32 |
| CFAT | 460 | 2.07 | 1.00 | 4.00 | 0.52 |
| EMD | 460 | 23.13 | 14.00 | 33.00 | 3.78 |
| GFW | 493 | 2.69 | 1.10 | 5.80 | 0.64 |
| FD | 468 | 16.67 | 12.90 | 30.00 | 3.06 |
| SS | 491 | 32.36 | 3.00 | 71.00 | 13.18 |
| WA | | | | | |
| EPWT | 1199 | 29.21 | 11.20 | 58.20 | 7.27 |
| PWWT | 1005 | 38.70 | 17.40 | 62.20 | 5.93 |
| YWT | 1008 | 43.32 | 21.20 | 70.20 | 7.50 |
| HWT | 821 | 49.03 | 49.03 | 72.20 | 7.56 |
| CFAT | 427 | 2.56 | 1.00 | 6.00 | 0.76 |
| EMD | 427 | 19.77 | 31.00 | 11.00 | 2.86 |
| GFW | 1005 | 4.54 | 1.70 | 7.80 | 0.84 |
| FD | 812 | 18.00 | 13.50 | 33.20 | 2.22 |
| SS | 806 | 31.71 | 5.00 | 68.00 | 14.68 |

**Table 5. Numbers of records per category (early post weaning (EPW), post weaning (PW), yearling (Y) and hogget (H) for production traits on INF ewes in WA and NSW.**

| | EPW | PW | Y | H |
|---|---|---|---|---|
| NSW | | | | |
| CFAT | | 304 | 156 | |
| EMD | | 304 | 156 | |
| GFW | | | 440 | 53 |
| FD | | | 441 | 27 |
| SS | | | 441 | 50 |
| WA | | | | |
| CFAT | 289 | 138 | | |
| EMD | 289 | 138 | | |
| GFW | | | 362 | 643 |
| FD | | 137 | 326 | 349 |
| SS | | 136 | 325 | 345 |

## 2.2 Statistical analysis

Data from NSW Activity 2 (methane data over different physiological states and methods) was analysed using general linear models within Minitab V 17. Fixed effects included method (RC and PAC), Batch (Physiological state at time of measurement, and for PACs run within day within batch), and during pregnancy, number of lambs in utero and during lactation, number of lambs at foot. Heritability and correlations for gas measurements (Activity 3) were estimated with ASReml (Gilmour *et al*. 2009). Univariate and bivariate animal repeatability models were run. In addition to the full pedigree, deep back-pedigree was provided by the INF with 18 founding genetic groups for these animals.

For the WA data, all data of follower ewes and lambs were combined. Significant fixed effects for $CH_4$, $CO_2$, $O_2$ and $CH_4/CO_2$ included group (Follower ewes autumn, follower ewes spring, follower lambs), date of birth and pregnancy status fitted within group, year of birth of the dam, and run (session) fitted within date of $CH_4$ measurement. The effect of the chamber was significant for $CO_2$ and $O_2$. For $O_2$ rear type was also fitted as significant effect. For liveweight at PAC measurement (lwt) fixed effects included group, date of birth and pregnancy status fitted within group and year of birth of the dam. Random effects tested

included animal id to estimate genetic animal variance and an identity matrix of animal id to estimate the permanent environmental variance. The permanent environmental effect was not significant for any of the traits, it was, however, included in the final model for all the other traits. Traits were standardised to a standard deviation of 1 for the bivariate analyses all gas measurements.

The same procedures were used for NSW data sets. In the NSW analysis, fixed effects were, Batch, Date, Run (within Day), Chamber (within Run), Date of Birth, Birth Type, Liveweight or feed intake. Animal was fitted as Random, and an identity matrix for animal effect (variance of permanent environmental effect) was created for the production traits CFAT (fat thickness at the c-site), EMD (depth of eye muscle at 12-13$^{th}$ rib) GFW (greasy fleece weight), FD (fibre diameter) and SS (Staple Strength). PACs were designed as a field measurement and in WA the sheep were measured off pasture to reflect methane emissions under commercial conditions. Variation in feed intake is the main contributor to variation in the gas measurements from PACs and a challenge is feed intake on pasture is unknown. To test the suitability of proxies to adjust for feed intake, models for all gas measurements were also run fitting live weight as covariate. In addition $CH_4$ was analysed by fitting $CO_2$ as a covariate, with the expectation that $CO_2$ provides additional information about both live weight and feed intake.

In the NSW data it was possible to compare the different PAC protocols (PAC0 and PAC1) (note that the PAC1 protocol is close to the field / pasture PAC protocol) with measurements in respiration chambers. Bivariate analyses were run using ASReml (Gilmour et al. 2009) to estimate correlations between the traits. Fixed effects as determined in previous analyses were fitted. In the models used to estimated correlations for CH4 measured in PAC0, PAC1 and RC, feed intake was fitted as a covariate.

Correlations between gas measurements of PAC1 in WA and PAC1 and RC in NSW were estimated also using ASReml (Gilmour et al. 2009). A bivariate sire model was fitted using fixed effects for the WA and NSW, respectively, as established in the previous analyses. Live weight was fitted as a proxy for feed intake because feed intake information was not available on the WA component. Residual variances were fixed to zero as the measurements did not share environmental variance and consequently phenotypic correlations could not be estimated.

To estimate variance components and genetic and phenotypic correlations for production and methane traits all data from NSW and WA were combined and bivariate sire models were run using ASReml (Gilmour et al. 2009) Fixed effects tested for production and methane traits included flock (NSW and WA), drop (2007 to 2014) and birth type (single, twins, or triplets). In addition for the PAC1 $CH_4$, date of measurement, batch (7 for NSW and 5 for WA), run (1 to 10), chamber and pregnancy status (dry, single or twin bearing) were tested as fixed effects. Batch, run and chamber were fitted within with date of measurement. Liveweight at PAC1 measurement was fitted as a covariate. For production traits age category (early post weaning, post weaning, yearling or hogget) was fitted as fixed effect and the weight appropriate for each measurement (early post weaning weight, post weaning weight, yearling or hogget weight) were fitted as covariates. Only significant effects were retained in the model.

#### (a) Genomic analysis

The objective of this work was to investigate the possibility that there were chromosomal regions associated with methane production. The initial step was to use genomic information to reconstruct pedigree and recapitulate the original genetic analysis derived from pedigree information (Robinson et al,, 2014). The data was then used for a genome wide screen to identify potential genomic regions of interest.

Ewes (total = 2455) from 5 flocks (Katanning WA n = 707, Cowra NSW n = 368, Armidale NSW n = 588, Trangie NSW n = 359, and Rutherglen VIC n = 433) with methane production (adjusted for Liveweight) measured in PACs and genotyped using the Illumina 12k sheep SNP chip were used for this analysis A single SNP regression was run for methane production that was used previously by Robinson et al. (2014) with the 12K SNP data. In a separate analysis genomic breeding values were estimated using a genomic relationship matrix (GRM) that was based on the 12K SNP information of the genotyped animals. To validate, that the subset of the genotyped animals is not biasing the results, heritabilities and estimated breeding values were firstly confirmed running the model of Robinson et al. (2014). A repeat measures model was used fitting dam, breed, flock, liveweight, year, time of measurement * flock, , flock * chamber, flock *day, flock * year, breed, flocks * day * run. There was different residual variance for each flock, and the data were log transformed to minimise variation between flock. In a second analysis, heritabilities were estimated using the subset of only the genotyped animals, fitting the same repeat measures model and effects and using pedigree. In a third analysis, genomic breeding values were estimated using the same model again, but fitting a GRM to describe the pedigree relationships.

# 3. Results

## 3.1 Activity 2 - NSW study

#### (a) Description of methane emissions over a full years breeding cycle

The measurement periods corresponding to differing physiological state of ewes; environment in which measurements were made (Animal House; AH v pasture); methods used for measurements and indicative level of feed intake within measurement period are shown in Figure 1. Methane production and yield of young growing, pregnant and dry ewes measured using RCs are shown in Table 6. Variation in methane yield during pregnancy is shown in Figure 3. Table 6 demonstrates an increasing trend for liveweight with age and feed intake with age and pregnancy status, which results in higher weights as dry 28 month old ewes, but lower FIDP and FIOD. Methane production increases from growing over pregnant to dry at 28 months of age. Methane yield is the lowest during pregnancy and highest in dry 28 month old ewes, which is graphically described in Figure 3.

**Table 6. The number (N) of ewes and trait means ± se mean for Liveweight, Feed intake day prior to RC measure (FIDP) feed intake on day of RC measure (FIOD) CH4 flux (mmol/min) measured over 22 hours, methane yield (MY, g $CH_4$/kg DMI calculated as 75% of intake on day of measure and 25% on day prior), Data shown are for ewes when growing at ~ 12 Months old (12 Mo), ~21 month old (during pregnancy also showing foetal number, 21 Mo Pregnant ) and ~28 months old when the ewes were non-pregnant – non lactating (28 Mo Dry).**

| | Growing 12 Mo | Pregnant 21 Mo | | | Dry 28 Mo |
|---|---|---|---|---|---|
| Foetal number | | 0 | 1 | 2 | |
| Trait | | | | | |
| N | 96 | 19 | 48 | 29 | 94 |
| Livewt (kg) | 50.5±0.79 | 47.8±1.33 | 54.7±1.07 | 59.9±1.53 | 60.3±0.93 |
| FIDP (g) | 1470±35 | 1400±42 | 1572±27 | 1637±40 | 1526±22 |
| FIOD (g) | $1081^{a}$±29 | $1283^{b}$±44 | $1506^{b}$±31 | $1541^{b}$±52 | $1344^{b}$±36 |
| $CH_4$ (mmol/min) | $0.917^{a}$±0.02 | $0.894^{b,x}$±0.02 | $0.979^{b,y}$±0.02 | $0.972^{bz}$±0.03 | $1.183^{c}$±0.02 |
| MY ($gCH_4$/kgDMI) | $20.19^{a}$±0.25 | $17.56^{b,x}$±0.27 | $16.52^{b,x}$±0.19 | $16.06^{b,y}$±0.33 | $21.62^{c}$±0.18 |

Effect of Age and Pregnancy status. Differences (P<0.05) for Time period described as a,b,c and x,y,z for Pregnancy status

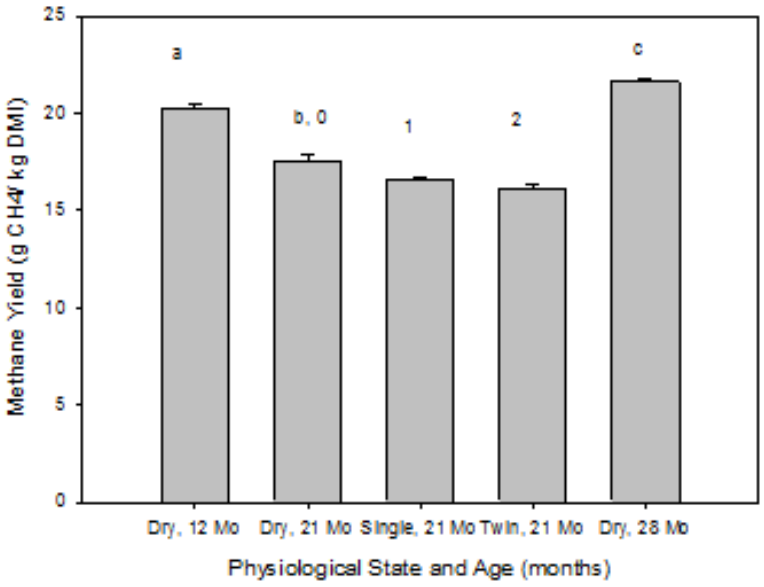


**Figure 3. The effect of physiological state (dry, 12 months of age, growing; non-pregnant, 21 months of age; pregnant with a single foetus, 21 months of age; pregnant with twin foetuses, 21 months of age; dry (non-pregnant, non-lactating, 28 months of age) on methane yield measured in Respiration Chambers. Values are means (g $CH_4$/kg DMI) ± se. a, b, c = effect of age / time of measurement and 0, 1, 2 effect of pregnancy at same time of measurement. Unlike symbols differ (P<0.05).**

Table 6 and Figure 4 demonstrates that variation in feed intake has a larger effect on total methane production than on variation in methane yield. Although there are systematic effects of pregnancy on methane yield (reduced by ~8%), there is no net reduction in total $CH_4$ production during pregnancy (in this data set) because the pregnant ewes ate more during the measurement period.

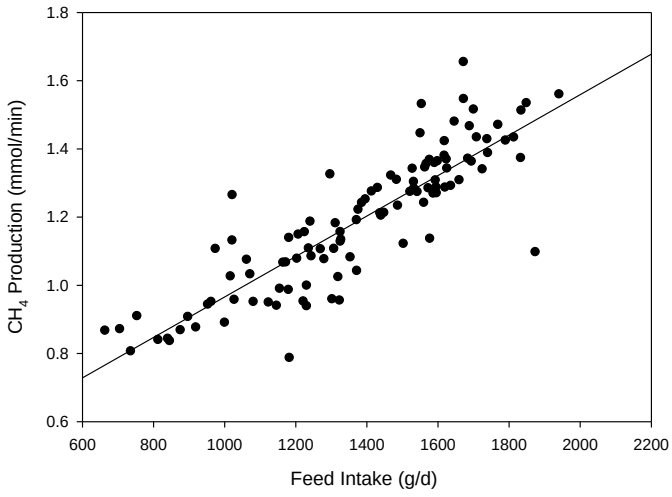


**Figure 4. Relationship between feed intake (g/d) and methane production (mmol/min) in dry ewes (non-pregnant or lactating).**

**(b) ii) Methane production in young growing, pregnant, lactating and dry ewes and effect of feed intake in PACs in both an animal house and pasture environment,**

In addition to measurement of $CH_4$ production in respiration chambers, measurements were also made in PACs. This allowed complementary data to be collected on the same animals in the animal house where intake was known and at pasture, where it was not possible to have a direct measure of feed intake. This was necessary because it was not practical to obtain data on $CH_4$ production in respiration chambers in lactation due to practical challenges in separating ewes from lambs, or co-locating lambs and ewes in respiration chambers).

To obtain information on potential proxies for feed intake, we used similar PAC protocols in the Animal House and at pasture, except we recorded feed intake prior to measurement in the Animal House (AH) . The best indirect estimate of feed intake in the AH was $CO_2$ flux and liveweight. We subsequently adjusted $CH_4$ measurements in the AH for feed intake and separately for liveweight and $CO_2$, and at pasture for liveweight and $CO_2$ to provide an indication of the likely intake of ewes at pasture. Figure 6shows methane production (mmol/min) adjusted for feed intake and for CO2 and Liveweight. It can be seen that adjustment for feed intake and for CO2 and liveweight provide similar estimates in the animal house (compare blue with red bars at each sample point). This provides some confidence that estimates of methane production from pasture fed animals (red bars only), after adjusting for CO2 and liveweight, reflect differences in feed intake

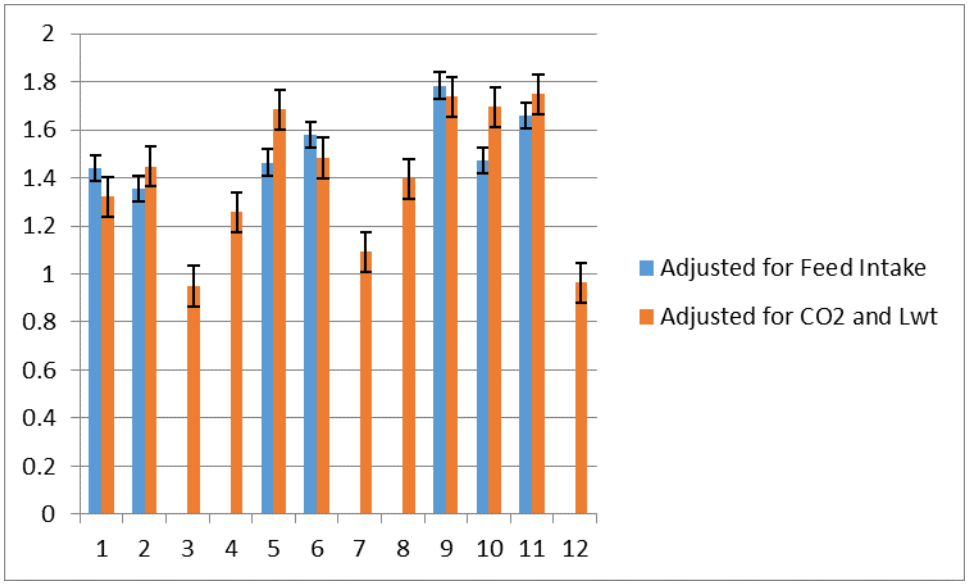


**Figure 5. Y axis = $CH_4$ (mmol/min) adjusted for feed intake (0.33*FIDP +0.67 FIOD, blue bars) or for Liveweight and CO2 production during $CH_4$ measurement. (red bars) X axis 1, 2 = 12 Month growing, 1 = Ad-lib, 2 = maintenance, 3,4 = 18 Months growing, pasture, 5, 6 = 21 Months pregnant 5 = 1.6 * Maintenance, 6 = ad-lib, 7, 8 = 24 Months, lactating, Pasture, 9,10,11 = 28 Months Dry 9 = 1.5*M, 10 = Ad-lib, 11= M, 12 = 30 Months, Dry, Pasture.**

The measurements of gas exchange in 24 month old lactating ewes on pasture (treatments 7, 8 in Figure 5) were broken down further to illustrate the effect of lactation on $CH_4$ and $CO_2$ output and $O_2$ uptake (Table 7). They show a significant increase in rate of $CH_4$ production in lactating compared to dry ewes or those that lambed but lost their lamb.

**Table 7. Least Square means for liveweight, $CO_2$ output, $O_2$ uptake and $CH_4$ emissions measured in PAC (adjusted for $CO_2$+$O_2$*RQ) by lactation status. Note intake was not known for lactating sheep because they were measured directly off-pasture.**

| Lactation Status | Liveweight (kg) | $CO_2$ output mmoles/min | $O_2$ uptake mmoles/min | $CH_4$ mmoles/min (adjusted) |
|---|---|---|---|---|
| Not lambed | $55.7_a$ | 18.07a | -17.93a | $1.431_a$ |
| Lambed and lost | $62.7_b$ | 19.71b | -20.01b | $1.420_a$ |
| Wet (lactating) | $61.9_b$ | 22.63c | -22.94c | $1.663_b$ |
| sed | 1.4 | 0.62 | 0.54 | 0.050 |

Means with different subscripts differ P<0.05

**Table 8. Sire progeny group rank for $CH_4$ emissions (adjusted for liveweight and feed intake) of dry ewes RC (1.5 * maintenance), PACs (maintenance, 1.5 * maintenance, ad-lib). The first section a) shows rankings from protocol where animals were fed up to time of measurement and includes RC data as a reference. The second b) shows rankings where animals were fed up to 1 hour prior to measurement. There were an average of 16 (range 19-29) progeny / sire.**

| Protocol / Intake | Sire M4 | Sire M5 | Sire MU1 | Sire W1 |
|---|---|---|---|---|
| a) *on feed* | | | | |
| RC (1.5*M) | 1 | 4 | 3 | 2 |
| PAC (M) | 1 | 4 | 2 | 3 |
| PAC (1.5*M) | 1 | 3 | 4 | 2 |
| PAC (Ad-lib) | 3 | 2 | 4 | 1 |
| b) *1hr off feed* | | | | |
| PAC (M) | 1 | 3 | 4 | 2 |
| PAC (1.5*M) | 1 | 4 | 3 | 2 |
| PAC (Ad-lib) | 3 | 2 | 4 | 1 |

The two protocols do not consistently rank sires the same across all treatments (i.e. there is a highly significant (P<0.001) method by treatment interaction) and a significant (P<0.05) treatment by sire interaction. There was no significant effect of sire across any levels of feed intake, due principally to re-ranking on ad-lib intake.

There is no consistency of ranking of sire progeny group on ad-lib intake (between RC, and between other levels of feed intake). This suggests that feeding behaviour in the period of ad-lib feeding prior to testing had a large effect on ranking. Perhaps, not surprisingly, there is a larger difference in intake on the day of measurement for the 1 hr off feed than the immediate off feed protocol on the ad-lib treatment. Sire rankings within 1.5 * maintenance and maintenance level of intake were generally consistent across methods (0 and 1 hr off feed prior to test), and similar to RC data.

These observations indicate that $CH_4$ production will reflect pattern of feed intake prior to measurement. For establishing a robust protocol for $CH_4$ measures in the field, this indicates that intake less than ad-lib (in excess of ability to eat) is likely to provide a more reliable ranking of sires. This was subsequently checked in the larger analysis of sheep information nucleus animals (described below).

Additional points:

1. Means of $CH_4$ production in PACs measured straight off feed (PAC0) and 1hr off feed (PAC1) are not significantly different. This is confirmed by subsequent measurements made in Activity 3 in the NSW measurements.
2. Intake on the day of measurement in PACs in animal house studies is dependent upon level of feeding. In particular, animals offered ad-lib (and which eat more / day) actually eat less than expected prior to measurement in PAC. This resulted in lack of sensitivity of PAC to detect differences in intake due to treatment. However, the PAC $CH_4$ and $CO_2$ production data reflects intake ($R^2$ 70-80%) prior to measurement.
3. PACs are less able to detect differences in physiological state than respiration chambers, because of larger error around measurement of $CH_4$ (and $CO_2$). This is more likely because of sampling errors due to the interaction between animal and the different measurement time in PACs compared to respiration chamber.

Implications for measurement of animals at pasture.

1. Production of $CH_4$ and $CO_2$ are highly correlated (r>0.85). Therefore, adjustment of $CH_4$ measured in PACs with measured $CO_2$ accounts for a substantial part of the variation in feed intake ($R^2$~70%). At the same time, $CH_4$ accounts for as much variation in feed intake as does $CO_2$.
2. This is important if an estimate of feed intake is required, for example, if we want to compare differences in methane yield between treatments. However, if, the trait intended for for genetic improvement is methane production (which includes information about feed intake) it is unlikely to be a consideration. See Robinson and Oddy (2016) and later in the report.

## 3.2 Activity 3 – NSW Genetic parameter estimates

A summary of heritability estimates of the sheep information nucleus ewes measured in NSW is shown in Table 9. Data shown was a combination of all data sets that were collected during Activity 3 in NSW. Not surprisingly the heritability of unadjusted traits was greater than after adjustment for liveweight (LWT) and for feed intake (FI), and lower after fitting the permanent environmental effect. For methane production, adjusting for feed intake provides the more reliable (plausible) estimate, adjustment for liveweight is next best (and potentially useful when feed intake data is not available). Repeat measures were useful to enable fitting of permanent environmental effect.

**Table 9:- Estimates of heritability for traits measured in NSW Sheep Information Nucleus ewes. Data were combined across all measurement protocols and adjusted for Feed Intake (adj FI), liveweight (adj LWT) or not adjusted for feed intake or liveweight (unadjusted) with and without correction for permanent environmental (PE) effects.**

| | Adj FI | Adj FI PE | Adj LWT | Adj LWT PE | Unadjusted | Unadjusted PE |
|---|---|---|---|---|---|---|
| $CH_4$ | 0.27 ± 0.03 | 0.26 ± 0.11 | 0.47 ± 0.03 | 0.19 ± 0.24 | 0.54 ± 0.03 | 0.31 ± 0.15 |
| $CO_2$ | 0.25 ± 0.03 | 0.06 ± 0.08 | 0.35 ± 0.03 | 0.00 ± 0.00 | 0.58 ± 0.02 | 0.43 ± 0.13 |
| $O_2$ | 0.44 ± 0.04 | 0.18 ± 0.15 | 0.52 ± 0.03 | 0.29 ± 0.35 | 0.64 ± 0.03 | 0.36 ± 0.18 |
| $CH_4$ / $CO_2$ | 0.20 ± 0.10 | 0.20 ± 0.10 | 0.24 ± 0.13 | 0.24 ± 0.12 | 0.37 ± 0.03 | 0.28 ± 0.12 |
| MY | | | | | 0.25 ± 0.03 | 0.08 ± 0.06 |

Note: Methane yield (MY) was not adjusted for feed intake or liveweight because the amount of feed was provided on a liveweight basis

## 3.3 Activity 3 - Heritabilities and genetic correlations for PAC and RC measurements for NSW data

Heritability estimates were moderate to high when data in NSW were adjusted with liveweight as a proxy for feed intake and analysed for a particular measurement technology (PAC0, PAC1 and RC) (Table 10). For PAC0 it was not possible to fit a permanent environmental effect due to a lack of repeat records and this has led to an overestimated heritability. Heritabilities were lower when methane production was adjusted for feed intake (Table 11). Feed intake accounted for an increased proportion of variation, which also decreased the heritability, but to have a consistent comparison of data throughout the report, and to compare withf the WA data where feed intake was not available, data were adjusted for liveweight. For PAC1 and RC, sufficient repeated records were available to fit a permanent environmental effect.

**Table 10. Heritability of methane production rate (ml/min - diagonal) adjusted for liveweight from 3 different measurement procedures / protocols and genetic correlation (below diagonal).**

| | CH4_PAC0 | CH4_PAC1* | CH4_RC* |
|---|---|---|---|
| CH4_PAC0 | 0.72 ± 0.06 | | |
| CH4_PAC1* | 0.91 ± 0.11 | 0.52 ± 0.12 | |
| CH4_RC* | 0.97 ± 0.14 | 1.00 ± 0.18 | 0.38 ± 0.11 |

*fitted permanent environmental effect

**Table 11. Heritability of methane production rate (ml/min - diagonal) adjusted for feed intake from 3 different measurement procedures / protocols and genetic correlation (below diagonal).**

| | CH4_PAC0* | CH4_PAC1 | CH4_RC* |
|---|---|---|---|
| CH4_PAC0* | 0.33 ± 0. 11 | | |
| CH4_PAC1 | 1.00 ± 0.24 | 0.27 ± 0.04 | |
| CH4_RC* | 1.00 ± 0.21 | 1.00 ± 0.30 | 0.37 ± 0.18 |

*fitted permanent environmental effect

**Table 12. Heritability of methane production rate (ml/min - diagonal) adjusted for liveweight from 3 different measurement procedures / protocols and genetic correlation (below diagonal).**

| | CO2_PAC0 | CO2_PAC1 | CH4_RC |
|---|---|---|---|
| CO2_PAC0 | 0.57 ± 0.08 | | |
| CO2_PAC1 | 0.87 ± 0.08 | 0.56 ± 0.07 | |
| CO2_RC | 0.56 ± 0.08 | 0.64 ± 0.07 | 0.63 ± 0.03 |

It was not possible to fit a permanent environmental effect for $CO_2$.

**Table 13. Heritability of carbon dioxide production rate (ml/min - diagonal) adjusted for feed intake from 3 different measurement procedures / protocols and genetic correlation (below diagonal).**

| | $CO_2$_PAC0 | $CO_2$_PAC1 | $CO_2$_RC* |
|---|---|---|---|
| $CO_2$_PAC0 | 0.41 ± 0.11 | | |
| $CO_2$_PAC1 | 0.87 ± 0.12 | 0.46 ± 0.05 | |
| $CO_2$_RC* | 0.55 ± 0.17 | 0.57 ± 0.21 | 0.34 ± 0.17 |

* Permanent environmental effect fitted

**Table 14. Heritabilities (on the diagonal) and genetic correlations (below the diagonal) for $O_2$ production measured with PAC0 and PAC1.**

| | $O_2$_PAC0 | $O_2$_PAC1* |
|---|---|---|
| $O_2$_PAC0 | 0.75 ± 0.05 | |
| $O_2$_PAC1* | 1.00 ± 0.06 | 0.54± 0.06 |

*fitted permanent environmental effect

The data in Tables 10 and 11 illustrates the high genetic correlation between measurements of methane production by the methods tested. Genetic correlation between PAC and RC methods is not different to 1, i.e. the methods measure essentially the same $CH_4$ trait. The genetic correlation between PAC and RC methods for measurement of $CO_2$ production rate is less favourable and indicates that $CO_2$ production rate in the PAC measurements is similar, but different to respiration chambers. The high genetic correlation for PAC protocols is confirmed by the genetic correlations for $O_2$ consumption. Oxygen data was not available from RCs. The resulting high correlations provide a sense of stability to measures from different measurement technologies. As will be seen later, one of the implications is that measurement of CH4 (and possibly $CO_2$ and $O_2$) is an indirect measure of feed intake.

## 3.4 Activity 3 - Heritability estimates from WA data (adjusted for liveweight)

Heritability estimates for gas measurements for PAC1 measurements were low to moderate. Data was adjusted for liveweight, same as for the heritabilities for the NSW data shown in Table 15. However, heritability estimates from the WA data are lower than for the NSW data. The most likely reason for this would be the larger number of records in the WA data set compared to the NSW data set, which is also reflected in the reduced standard errors of the estimates. Still, heritabilities are somewhat higher than estimates previously reported for PAC measurements (Robinson *et al.* 2014, Goopy *et al.* 2015). Heritabilities from

respiration chambers for methane production in g/day of $h^2$ = 0.29 and methane yield of $h^2$ = 0.13 were reported by Pinares-Patiño *et al.* (2013).

**Table 15. Heritabilities and variance components for WA data from PAC1 (adjusted for lwt).**

| | $V_P$ | $V_G$ | $h^2$ |
|---|---|---|---|
| $CH_4$ | 9.70 | 1.94 | 0.20 ± 0.05 |
| $O_2$ | 1551.60 | 453.86 | 0.19 ± 0.06 |
| $CO_2$ | 1039.50 | 306.55 | 0.29 ± 0.06 |
| $CH_4/CO_2$ | 0.02 | 0.002 | 0.09 ± 0.05 |

### (a) Activity 3 - Comparison of gas measurements recorded with different technologies in WA and NSW

The genetic correlations for gas measurements taken in two different environments were high across PAC1 and RC, with the exception of $CO_2$ between PAC1 in WA and RC in NSW, which had only a moderate correlation The most important results is that this analysis shows that estimates of methane adj lwt NSW and WA were essentially the genetically the same trait. This means that groups of same sire in have similar rank. Although the se of rg is high, this most likely reflects the relatively small number of progeny / sire group in each location (NSW range 1-9, average 2.8; WA 1-16 average 3.6 progeny / sire). This analysis provides some confidence that combining NSW and WA data to estimate genetic correlations between methane and production traits will be useful.

**Table 16. Genetic correlations of gas measurements obtained with the same and different measurement technology applied in NSW and WA – adjusted for lwt.**

| NSW | WA | $r_g$ |
|---|---|---|
| $CH_4$_PAC1 | $CH_4$_PAC1 | 0.99 ± 0.42 |
| $CO_2$_PAC1 | $CO_2$_PAC1 | 0.75 ± 0.52 |
| $O_2$_PAC1 | $O_2$_PAC1 | 1.03 ± 0.40 |
| $CH_4$_RC | $CH_4$_PAC1 | 0.93 ± 0.89 |
| $CO_2$_RC | $CO_2$_PAC1 | 0.41 ± 0.22 |

## 3.5 Activity 4 - Genetic correlations between CH4 and production data from combined data (WA and NSW)

The heritabilities for the production traits ranged from low to very high, with the heritability for GFW being the highest ($h^2$=0.79 ± 0.10). With the exception of the heritability of GFW, all other estimates are within the range of reported estimates (Safari and Fogarty, 2003). Other analyses of the INF data found also very high correlations for GFW (J. Smith, CSIRO Agriculture and Food, not published). The standard errors are high, as can be expected considering the modest number of records available for analysis.

**Table 17. Genetic ($V_G$) and phenotypic ($V_P$) variance components, heritabilities ($h^2$) and genetic ($r_G$) and phenotypic correlations ($r_p$) with PAC1 CH4 (all traits adjusted for Liveweight).**

| | $V_p$ | $V_g$ | $h^2$ | $r_g$ | $r_p$ |
|---|---|---|---|---|---|
| CFAT | 0.23 | 0.05 | 0.20 ± 0.10 | -0.11 ± 0.28 | -0.33 ± 0.15 |
| EMD | 5.04 | 2.29 | 0.45 ± 0.13 | -0.35 ± 0.23 | -0.27 ± 0.11 |
| GFW | 0.40 | 0.32 | 0.79 ± 0.10 | 0.25 ± 0.15 | 0.13 ± 0.05 |
| FD | 2.66 | 1.51 | 0.57 ± 0.10 | -0.09 ± 0.18 | 0.06 ± 0.06 |
| SS | 85.30 | 17.73 | 0.20 ± 0.09 | -0.03 ± 0.25 | -0.02 ± 0.10 |

The genetic and phenotypic correlations between methane and production traits that resulted from the bivariate analysis are only an indication of the relationship between the traits due to the large standard errors that are associated in particular with the genetic correlations. The phenotypic correlations with live animal scan traits are moderately negative and significantly different from zero. The phenotypic relationships with GFW and FD are lowly positive and close to zero for SS. The same direction of the phenotypic relationships was observed in the genetic correlations with negative correlations with scan, positive with GFW and a correlation close to zero for SS. The genetic correlation with FD is negative, but is not different from zero.

## 3.6 Activity 4 - Towards development of genomic breeding values for methane traits

Apart from direct measurement of methane, it may be possible to estimate genomic breeding values for methane traits and use these in selecting animals from which to breed.

**Table 18. Heritability estimates derived from pedigree and genomic information. (JAS = estimates as reported by Robinson et al. (2014), All Animals = confirming the model used in the analysis of Robinson et al. (2014), Genotyped = using pedigree for genotyped animals only, GRM = using genomic relationship matrix for genotyped animals only).**

| | JAS | All Animals | Genotyped | GRM |
|---|---|---|---|---|
| Cowra | 0.07 | 0.05 | 0.064 | 0.048 |
| Katanning | 0.12 | 0.08 | 0.097 | 0.071 |
| Kirby | 0.11 | 0.11 | 0.134 | 0.098 |
| Rutherglen | 0.04 | 0.03 | 0.044 | 0.032 |
| Trangie | 0.11 | 0.07 | 0.044 | 0.062 |

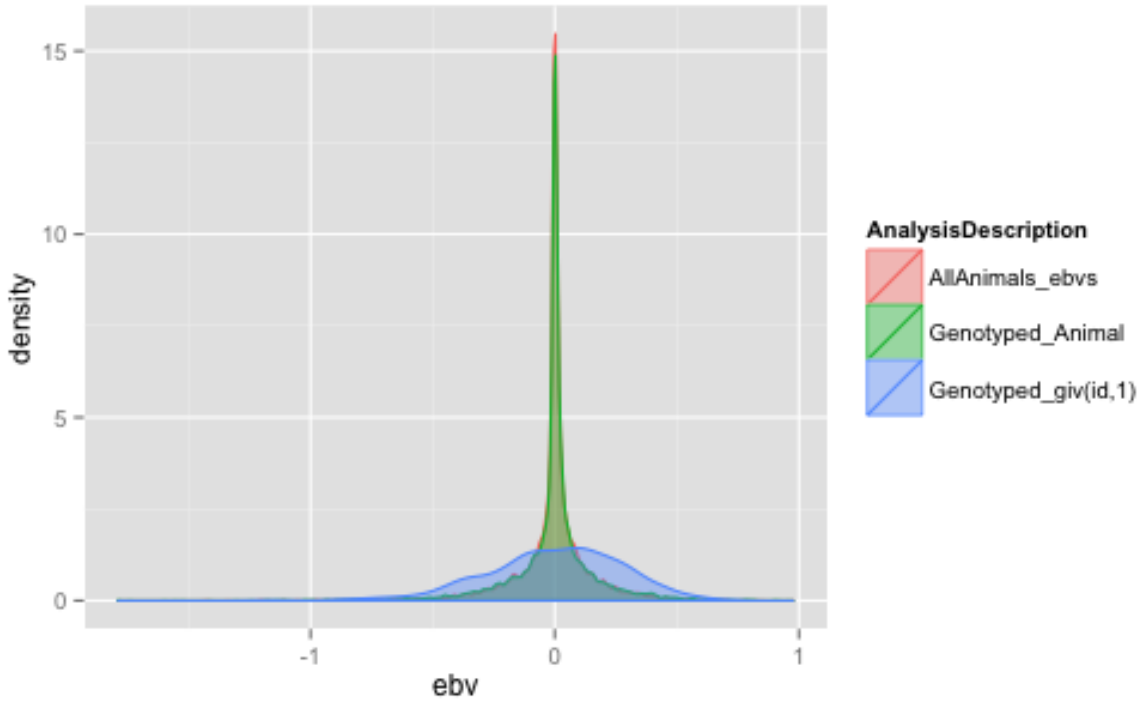


**Figure 6. Density distribution of EBVs estimated from pedigree using all animals (AllAnimal_EBVs) using genotyped animals only (Genotyped_Anjmal) and GEBVs estimated using a GRM (Genotyped_giv(id,1)).**

Figure 6 illustrates that the estimated breeding values (EBVs) for methane adj liveweight derived from analysis of pedigree data are strongly related to those computed using genomic derived data (genomic relationship matrix: GRM. The distribution of genomic breeding values (GEBV) generated by using a GRM is wider, but predictions were based on 12K SNP chip information only.

The size of marker effects across the genome (Figure 7) does not support the concept that methane production is under the control of a single (or a limited number of) genetic loci. This indicates that there is more likely to be many loci contributing to variation in methane production in sheep. These results indicate that procedures utilising many markers (and development of genomic breeding values) are likely to be required to implement genomic based breeding solutions for methane traits.

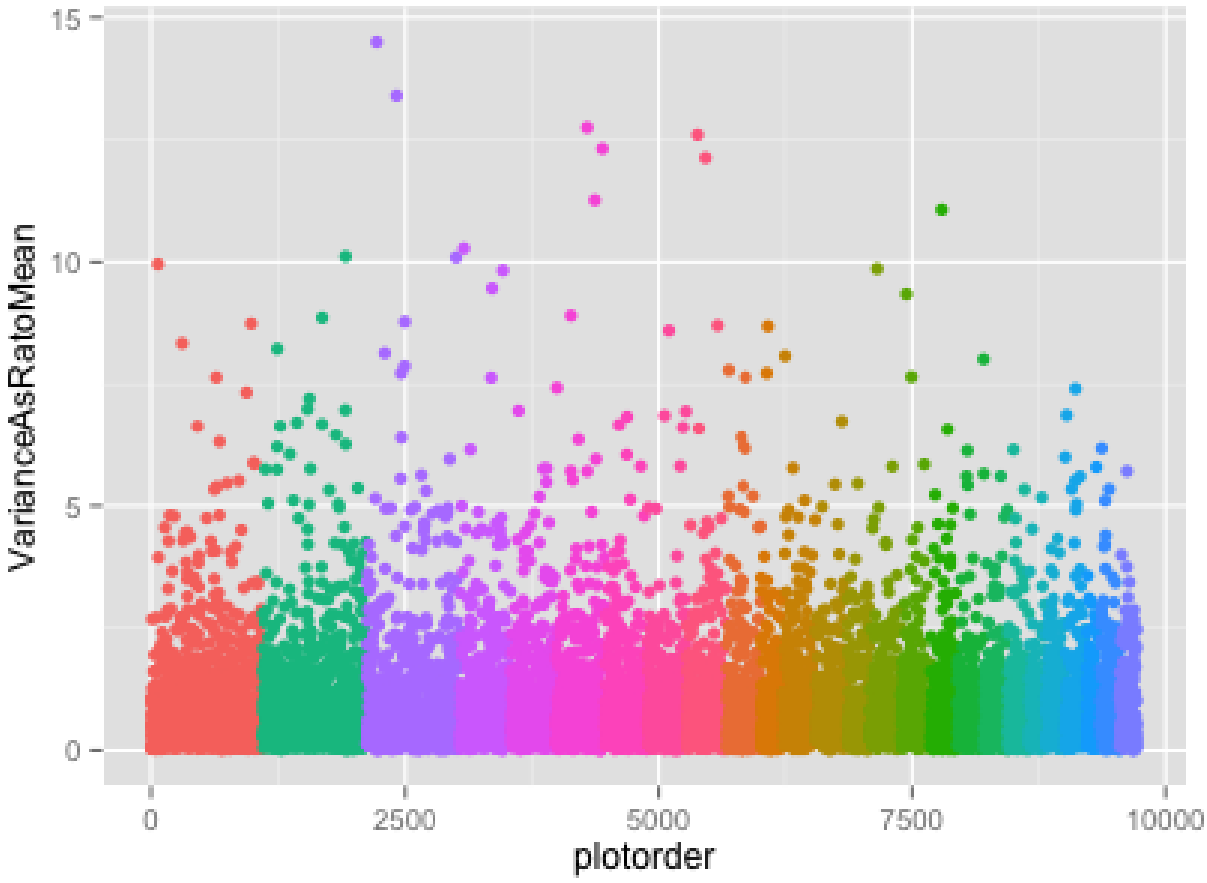


**Figure 7. Genetic variance in methane production explained by single SNP (in %). The colours of the X-axis indicate chromosomal number.**

## 3.7 Activity 4 - Can the sheep industry breed low methane sheep without compromising productivity?

The work underpinning this component of the report has been published in Journal of Animal Science doi:10.2527/jas.2016-0503

The report below consists of an abstract of the published paper and a copy of the appendix to that paper which contains the synthesis of data used to generate the genetic parameters used to conduct the simulations.

***Abstract of Paper "Benefits of including methane measurements in selection strategies". DL Robinson and VH Oddy, 2016 Journal of Animal Science doi:10.2527/jas.2016-0503***

Estimates of genetic/phenotypic covariances and economic values for slaughter weight, growth, feed intake and efficiency, and three potential methane traits were compiled to explore the effect of incorporating methane measurements in breeding objectives for cattle and meat sheep. The cost of methane emissions was assumed to be A) zero, B) A$476/tonne (based on $14/tonne $CO_2$-equivalent and methane's 100-year Global Warming Potential, GWP of 34) and C) A$2,580/tonne ($30/tonne $CO_2$-equivalent combined with methane's 20-year GWP of 86). The methane traits were: methane yield (MY, methane production divided by feed intake, based on measurements over 1 d in respiration chambers), or short-term measurements of methane production adjusted for liveweight (MPadjWt) in grazing animals, e.g. 40-60 min methane measurements in portable accumulation chambers (PAC) on 1 or 3 occasions, or measurements for 1 wk using a Greenfeed Emissions Monitor (GEM) on 1 or 3 occasions. Feed costs included the cost of maintaining the breeding herd and growth from weaning to slaughter. Sheep were assumed to be grown and finished on pasture (A$50/tonne DM). Feed costs for cattle included 365 days on pasture for the breeding herd, and averages of 200 d post-weaning grow-out on pasture and 100 d feedlot-finishing. The greatest benefit of including methane in the breeding objective for both sheep and cattle was as a proxy for feed intake. For cattle, 3 GEM measurements were estimated to increase profit from 1 round of selection in scenario A (no payment for methane) by A$6.24/head (from A$20.69 to A$26.93) due to reduced feed costs relative to gains in slaughter weight, and by A$7.16 and A$12.09/head respectively for scenarios B and C, which have payments for reduced methane emissions. For sheep, the improvements were more modest. Returns from 1 round of selection (no methane measurements) were A$5.06 (scenario A), A$4.85 (B) and A$3.89 (C), compared to A$5.26 (scenario A), A$5.12 (B) and A$4.72 (C) for 1 round of selection with 3 PAC measurements. Including MY in the selection index was less profitable because it did not reduce feed costs relative to weight gain. Consequently, for strategies measuring MY but not MPadjWt (or other estimate of feed intake in the production environment) proportionately greater emphasis was placed on increasing slaughter weight, and as a result, the decreases in methane emissions per head, and per unit of feed intake were smaller than for strategies that measured MPadjWt.

## 3.8 Activity 5 - Samples and data derived from this study made available to the Host Control project within the Rumen PanGenome Project

Samples of rumen fluid and faeces were collected from the 96 ewes used in the first part of this study. Samples were collected at *ad-lib* and maintenance intake while the ewes were growing (12 months of age) pregnant (21 months of age) and again when dry (28 months). Methane (and CO2) measurements were made using both respiration chambers and PACs as described above. Volume of the reticulo-rumen (including of the reticulum, dorsal and ventral sacs) was measured on CT scanned images within a few days of measurement in respiration chambers. In addition to measurement of volume, an assessment of the contents of the reticulo rumen and individual sacs was made similar to that described by Bain et al, 2014. This allowed estimation of gas, mixed particulate and liquid phases of the contents. DNA was extracted from blood samples collected from all ewes and genotyped on the Illumina 600k Sheep SNP chip.

Volatile fatty acid composition in rumen fluid was estimated by gas liquid chromatography (see Bond et al, 2016 – submitted), microbial composition was determined by procedures

described by Denman et al (see report "Host control of methane emissions from sheep" Rumen metabolite concentration was determined using proton spin NMR as follows.. $^{1}H$ NMR spectra were acquired at 298K in 3 mm tubes on a Bruker Avance 900 NMR spectrometer with CryoProbe using a SampleJet (96 tube racks) for sample introduction. Samples were maintained at 4$^{o}$C in the SampleJet prior to introduction into the probe and an equilibration time of 6 minutes was allowed before commencement of acquisition. Standard Bruker pulse sequences were used (noesypr1d). NMR spectra were processed with Topspin 3.2 software, using multiplication by a sine bell, shifted by 90$^{o}$, prior to Fourier transformation and manual phase correction. Spectra were referenced to internal 4, 4-dimethyl-4-silapentane-1-sulfonic acid (DSS). Metabolite concentrations were determined by integration relative to the integral of the internal standard, difluorotrimethylsilylmethylphosphonic acid (DFTMP) (386µM). 64 of the 96 ewes used for the above study were used in the phenotype study that underpinned the Host control of methane production from sheep project (Bond et al, 2016).

The data from the first phase of this project and the above samples are an integral part of the Host control project. The results of measurements conducted in that project will be reported therein.

# 4. Discussion

The significant insights from this work and presented in the milestone and whole of project progress statement tables above are discussed below.

## 4.1 Measurement of methane emissions from sheep for genetic evaluation purposes

This work has clearly shown that measurement of $CH_4$ production by sheep is best done while the animals are neither pregnant nor lactating, because of specific effects of these physiological states on methane emissions. These changes are most likely invoked as alteration in feed intake and / or changes in flow rate through the rumen which can alter the relationship between methane production and dry matter intake.

The extensive comparative study of RC and PAC data enabled a clear demonstration that for the purposes of genetic evaluation both methods are valid measurement systems. If animals are eating the same feed (quantity and quality) the genetic correlations between measurements of methane production using the "technically best" technique (respiration chambers) and measurements made in portable chambers are no different to 1. This offers industry confidence that the cheaper high throughput protocol available through the use of PACs will not mislead industry in selection of high and low emitting sheep.

However, the high correlation between methane production and feed intake, almost certainly means that measurement of $CH_4$ in the field (using PACs) is also a proxy measure of feed intake. This has implications for choice of trait. Because of that strong relationship we, and others (National Greenhouse Gases Inventory Procedures 2014; Amer and Fennessy, 2012; Arthur et al. 2016) initially considered methane yield the trait of choice. In practice this meant that not only a measure of $CH_4$ was required, but also one for feed intake, on the same animal. Measurement of feed intake is a hard problem, even under controlled

circumstances such as RC, because individual animal behaviour also affects the amount eaten. However, we were already aware that the high correlation between intake and CH4 production meant that these traits were inextricably coupled. In our work we spent considerable time trying to devise proxy measures of intake so we could estimate methane yield. These included simultaneous measures of $CO_2$ production and $O_2$ consumption at the time $CH_4$ measures were made, use of rumen or plasma VFA, and as a crude approximation, liveweight of the animal.

It wasn't until we compiled all the available data on genetic correlations between methane traits, feed intake and production traits, and include them in a simple selection index that it became clear to us that methane yield had a number of limitations as a trait of choice for genetic evaluation. The simulations (results and discussion below) show that using MY in a selection index neither improves (reduces) methane production or improves productivity (output per unit feed input), but using direct measures of methane improve both. The reason using MY in a selection index is not profitable is because selection on MY has no effect on feed intake, largely due to MY being the ratio of methane production to feed intake. This paradoxical result was not obvious to us at the commencement of the study. The basis for the observation and implications are discussed in more detail below.

## 4.2 Using methane measurements in breeding objectives

*Abstract of Paper "Benefits of including methane measurements in selection strategies". DL Robinson and VH Oddy, 2016 Journal of Animal Science doi:10.2527/jas.2016-0503*

Estimates of genetic/phenotypic covariances and economic values for slaughter weight, growth, feed intake and efficiency, and three potential methane traits were compiled to explore the effect of incorporating methane measurements in breeding objectives for cattle and meat sheep. The cost of methane emissions was assumed to be A) zero, B) A$476/tonne (based on $14/tonne $CO_2$-equivalent and methane's 100-year Global Warming Potential, GWP of 34) and C) A$2,580/tonne ($30/tonne $CO_2$-equivalent combined with methane's 20-year GWP of 86). The methane traits were: methane yield (MY, methane production divided by feed intake, based on measurements over 1 d in respiration chambers), or short-term measurements of methane production adjusted for liveweight (MPadjWt) in grazing animals, e.g. 40-60 min methane measurements in portable accumulation chambers (PAC) on 1 or 3 occasions, or measurements for 1 wk using a Greenfeed Emissions Monitor (GEM) on 1 or 3 occasions. Feed costs included the cost of maintaining the breeding herd and growth from weaning to slaughter. Sheep were assumed to be grown and finished on pasture (A$50/tonne DM). Feed costs for cattle included 365 days on pasture for the breeding herd, and averages of 200 d post-weaning grow-out on pasture and 100 d feedlot-finishing. The greatest benefit of including methane in the breeding objective for both sheep and cattle was as a proxy for feed intake. For cattle, 3 GEM measurements were estimated to increase profit from 1 round of selection in scenario A (no payment for methane) by A$6.24/head (from A$20.69 to A$26.93) due to reduced feed costs relative to gains in slaughter weight, and by A$7.16 and A$12.09/head respectively for scenarios B and C, which have payments for reduced methane emissions. For sheep, the improvements were more modest. Returns from 1 round of selection (no methane measurements) were A$5.06 (scenario A), A$4.85 (B) and A$3.89 (C), compared to A$5.26 (scenario A), A$5.12 (B) and A$4.72 (C) for 1 round of selection with 3 PAC measurements. Including MY in the selection index was less profitable because it did not reduce feed costs relative to weight gain. Consequently, for strategies

measuring MY but not MPadjWt (or other estimate of feed intake in the production environment) proportionately greater emphasis was placed on increasing slaughter weight, and as a result, the decreases in methane emissions per head, and per unit of feed intake were smaller than for strategies that measured MPadjWt.

The economic analyses above suggest that methane emissions measured for 40-60 min in Portable Accumulation Chambers, or over 1 wk using the Greenfeed Emissions Monitor system are useful traits to consider for inclusion in the breeding objective. Depending on costs and benefits, it could also be worthwhile to repeat the measurements, ideally after an interval of at least 2 wk, or at a different time of year. There are obvious benefits in measuring feed intake for research purposes and to improve the accuracy of estimated genetic and phenotypic covariance matrices. However, when it is not practical or cost effective to measure feed intake, methane emissions can be used as a proxy for feed eaten over the previous 1-3 d. Even at the highest plausible cost of methane emissions (A$2,580/tonne, calculated using methane's 20-year GWP of 86 $CO_2$-eq cost of $30/tonne) the economic benefits achieved by improved feed efficiency are greater than those from reducing methane emissions. With respect to utility of PAC measures acting as a proxy for feed intake, we have further evaluated the relationship between long and short term measures of feed intake and CH4 production D.L. Robinson, M. Cameron, A. J. Donaldson, S. Dominik and V.H. Oddy "One hour portable chamber methane measurements are repeatable and provide useful information on feed intake and efficiency" (in review Journal of Animal Science)

Feed intake (**FI**), liveweight (**LW**) and weight gain were recorded over 31 days in 96 12-month old ewes (progeny of 4 sires) given ad lib access to lucerne/oat chaffed hay, together with methane and $CO_2$ emissions measured for 40-60 min in portable accumulation chambers (**PAC**) and in respiration chambers (**RC**) over 22 h. RC testing increased the variability of FI on the test day and depressed the amount eaten from an average of 1384 to 1062 g/d; RC FI depression increased by 0.63 ± 0.24 percentage points for every kg of additional LW. PAC measurements were quite repeatable before (rpt = 0.76 for $CH_4$, 0.81 for $CO_2$) and moderately repeatable after (rpt = 0.47 for $CH_4$, 0.43 for $CO_2$) adjusting for weight and weight gain. Daily feed intake measurements had similar repeatability (0.76 before, 0.42 after adjustment for weight and weight gain). PAC measurements were highly correlated with mean 31-day feed intake (**mFI**, r = 0.81 for both $CH_4$ and $CO_2$). After adjustment for weight and weight gain, they were moderately correlated with residual feed intake (**RFI**, r = 0.37 for $CH_4$, 0.31 for $CO_2$). The $CH_4$:$CO_2$ ratio was also significantly correlated with mFI (mean daily feed intake, r = 0.52). After pregnancy and lactation, 91 of the ewes had repeat PAC measurements at 2 years of age when given ad lib access to the same feed. Correlations with 2012 PAC measurements were 0.64 ($CH_4$) and 0.75 ($CO_2$). After adjusting PAC measurements in 2014 for LW, correlations with RFI in 2012 were 0.34 ($CH_4$) and 0.33 ($CO_2$), with a clear, almost linear relationship between sire means for RFI in 2012 and PAC $CH_4$ adjusted for LW in 2014. These results suggest that PAC tests under similar feeding conditions are repeatable over an extended time period and can provide useful information on feed intake and efficiency as well as methane emissions.

Feed costs represent a substantial proportion of the variable costs of both feedlot and pasture production systems and are a major determinant of profitability (Goddard et al., 2011; Hoque and Suzuki, 2009). There are many different aspects to complex efficiency traits such as RFI, which was noted to have repeatability across diets of 0.33 to 0.67 (Basarab

et al., 2013). For example, improved RFI in Nellore steers was thought to be associated with lower degrees of activity and responsiveness to stress and lower losses of dietary energy as methane (Gomes et al., 2013). Herd et al. (2011) reported that heifers selected in a post-weaning feed efficiency test did not have improved efficiency when feed intake was restricted, although they were superior in size and efficiency as cows on medium-quality pasture or on unrestricted pellet feeding. This suggests that it would be advantageous to have information on feed intake and efficiency under all pasture conditions relevant for livestock production systems, enabling breeding objective software to be utilized to select the most appropriate animals for the environment in which they will be used.

As a general principle, if a test with half the measurement error costs twice as much, and it would cost too much to use the more accurate test on all animals, using the cheaper measurement on twice as many animals will generate greater genetic gain for the desired production environment than using the more expensive test. The development of a practical measure of feed intake in a pasture-based environment was described as a serious challenge (Greenwood et al., 2014, Cottle, 2013). It is now common for accredited ultrasound scanners to travel to breeding herds to measure muscle area, fat depths and marbling (Robinson et al., 1993). If the cost of providing a service to measure methane in PACs is a similar order of magnitude, incorporating the measurements into a genetic selection index has the potential to improve efficiency whenever useful genetic variation exists in this trait and at the same time reduce methane emissions intensity.

## 4.3 Genetic and phenotypic correlations with production traits

The association between live weight and methane production is highly positive. It was surprising to find negative phenotypic and genetic correlations between CFAT and EMD from ultrasound scanning with methane production. Live weight was fitted in the statistical model, which would mean that the negative association between methane and body composition traits relates to the comparatively small component of fat and eye muscle depth (or area) that is independent of live weight. Wool quality appears to be independent of methane production, but GFW showed a positive genetic and phenotypic correlation with methane production. In the analysis we could not adjust methane production for feed intake because the WA component of the data set did not have feed intake records. It is possible that the increase in GFW with increase methane production is associated with the remaining effect of feed intake on the methane measurement.

The results of this study do not lead to strong conclusions of how the inclusion of methane production might affect the major production traits in Merino sheep, but it might affect carcase traits positively and wool production negatively. This is consistent with results of Pinares-patino *et al.* (2013) who showed a positive (although close to zero) genetic correlation between wool production and daily methane production and a negative genetic correlation between wool production and methane yield. Overall, the effect on overall profit depends on the economic value that is placed on methane production, and the production traits when they are integrated into a selection index.

## 4.4 Implications for industry and policy makers

This research clearly shows that methane traits are heritable. This opens the possibility that selection of breeding animals for lower methane emissions is technically possible. Measurement of methane emissions from animals in such a way as to include the information in genetic evaluation programs is now possible. During the course of this project it became clear that the trait to measure is methane production (with appropriate adjustment for weight or feed intake) in animals in a grazing environment. The results show that in an environment where feed intake is controlled and known, measurement in the "gold standard" respiration chambers is highly genetically correlated with measurements made in portable chambers. The high correlations between the different measurement technologies, as evaluated within the NSW data, provides some confidence that methodology and protocols used to measure methane are robust, Furthermore, the observation that the high genetic correlation between measurements made in NSW and in WA was high also reflects little genotype x environment interaction for methane production across these two locations and that re-ranking of sires in their genetic value would be minimal. .

Subsequent demonstration that greater genetic progress towards improving profit and reducing methane emissions can be made by using the trait methane adjusted for liveweight rather than methane yield (methane / feed intake) provides an avenue to include measurements of methane production into genetic evaluation programs. The strong genetic correlation between methane production and feed intake is seen to underpin the advantage of measurement of methane in part as a substitute for measurement of feed intake. Simulation of effect of inclusion of different ways of expressing methane (and of measurement of methane) shows that there are production benefits (in terms of reduced feed intake to obtain the same level of productivity) before there are substantial impact on methane production. This is independent of any carbon pricing mechanism, but accelerated as the carbon price increases. Even at a higher than anticipated carbon price ($30 tonne $CO_2$-e and GWP of $CH_4$ = 86) the major benefit of including methane adjusted for liveweight in a selection index is its effect on reducing feed intake (Robinson & Oddy, 2016).

**The implications of this result is that there is no financial impediment to including measurements of $CH_4$ in a selection index now. If and when a carbon pricing mechanism is introduced the advantage will grow.**

From a policy perspective, this means that the feared negative impact of carbon pricing on animal production will in part be negated by the benefits available by proactively including $CH_4$ in a breeding index even when no carbon pricing mechanism is in place. This of course needs further refinement, but such refinement will not be possible until substantial numbers of direct measurements of $CH_4$ are made in the national flock. For this to happen measurement of $CH_4$ (and or feed intake) will need to be included in a functional breeding objective that has been accepted by industry. Discussions with Sheep Genetics indicate acceptance by industry will be driven by productivity gains arising from $CH_4$ measurement being used as a proxy for measurement of feed intake, rather than immediate concern for $CH_4$ emissions in the absence of any carbon pricing mechanism. The simulation of impact of including methane production in a breeding objective indicates that continued selection for productivity without a Carbon price will only increase $CH_4$ emissions even if $CH_4$ is measured. The rate of increase in total $CH_4$ emissions will not decrease without a Carbon price, and will

only lead to a net reduction in methane production at high feed costs, or at a Carbon price unlikely to be implemented in the near time.

It may be possible to use genomic predictions for methane production (and feed intake). It is unlikely that such complex traits (as methane production and / or feed intake) are controlled by just a handful of genes and therefore the development of a genetic test is unlikely. However, it is still possible given the increasing use of SNP genotyping throughout the breeding sector of the sheep (and cattle) industry that a blend of direct measurement to obtain data on influential replacement animals, and genomic information on all relevant breeding animals will be acceptable and can be backed on the genotyping efforts for other traits.

## 4.5 Alternative methods to reduce $CH_4$ emissions from sheep`

Because of the way sheep are managed in Australia, there is no practical way to include supplements that reduce $CH_4$ into sheep on a year round basis. This points to implementing a process to permanently change the animal through either vaccination (not yet available) or selective breeding (shown to be possible) or a combination approach. The analysis conducted in this report illustrates that even with no price on methane, the advantage of including measurement of methane production in a breeding objective (as a proxy for feed intake) increases profit from selection for production and reduces the rate of increase in methane production. There is no reason to believe that in the event that successful vaccination technologies, or longer term inhibition strategies cannot be used in conjunction with a breeding program. In the absence of such strategies, it would be churlish to reject the productivity gain and reduced rate of methane production available from implementing a breeding strategy to reduce methane, even in the absence of a price on carbon or mechanism to recover credits from reducing agricultural emissions.

Contrast the results from this research with those in Amer & Fennessy (2012) who advocated development of a process to select for low(er) methane yield (i.e. g $CH_4$/kg feed eaten), and Fennessy, Byrne & Proctor (2015) who modelled the industry benefit of selection for lower methane yield (and found the benefit to be quite small, as do Robinson & Oddy, 2016). However, these studies were constrained by lack of available information on the genetic and phenotypic correlations between methane and production traits. Where such data was available, Robinson & Oddy 2016, demonstrated that inclusion of daily $CH_4$ measures in a selection index improved profit through their genetic relationship with feed intake, and that using the trait of methane yield had little effect on profit or on total $CH_4$ production. It also showed that by using measurement of DMP or MP adjusted for liveweight in a portable chamber or Greenfeed emissions monitor had a greater impact on methane yield than direct measure of MY in a RC, as a consequence of the strong genetic correlation between $CH_4$ production and feed intake. This result is markedly different to the opinion offered to MLA (B.CCH.1075) by Amer and Fennessy (2012) vis “Simple selection criteria based on gross methane output are unlikely to contribute to a viable methane reduction business case because of inherent and unfavourable associations between gross methane yield and productivity.”

An analysis of the potential benefit of breeding for low methane emissions (in the Australian Beef industry) “Estimating the potential impact of different mitigation strategies to reduce methane output from beef cattle (MLA Project B.CCH.6133)” Peter Fennessy, Tim Byrne &

Luke Proctor; AbacusBio Limited, 31 May 2015, was commissioned by MLA and used in the National Needs and Gaps Analysis arising from the National Livestock Methane Program. This report showed that using methane yield as a selection criteria resulted in a small (neglible) benefit of breeding to reduce methane production because of the small reduction in methane production and lack of gain in productivity. This result is similar to that obtained by Robinson and Oddy (2016) using methane yield as a trait for selection. Moreover, that report did not have access to all the genetic correlations between methane and production traits, in particular relationship between methane, intake and residual feed intake.

The modelling used in this report (B.CCH.6133) was based on a 3-trait genetic model (600-day weight, mature cow weight and days to calving). Feed intake was calculated from other parameters – no allowance was made for genetic variation in feed efficiency. Methane was assumed to be measured as methane yield (**MY**); there was no attempt to use methane measurements to reduce feed requirements. Instead, Fennessy et al. (2015) argued that a charge for carbon emissions is effectively a tax on the production of beef because it is a charge on feed consumed by the cattle. According to Fennessy et al. (2015), a $CO_2$-eq price of $25/tonne is expected to increase the price of beef by about $0.44 per kg carcass weight.

The results reported by Fennessy et al. (2015) are broadly similar to the results for MY of Robinson and Oddy (2016) in that MY measurements do not improve profitability in the absence of a carbon tax. Even at modest carbon prices, a large proportion of the improvement in profitability is due to improvement in other traits. In the analysis of Fennessy et al. (2015), with a carbon price of $25/tonne, 89.2% of the improvement in the economic response is from weight at slaughter, mature cow weight and fertility; only 10.8% is due to reductions in MY. The reduction in $CH_4$ emissions is a modest 0.67 kg $CH_4$ per cow mated per year with a stated economic value of $0.02. (This is presumably the difference between selection with and without methane measurements; 0.67 (kg $CH_4$) x $0.625 (price per kg CH4) = $0.08, which would have to be offset against the cost of the reduced responses in the other traits).

A different approach was taken by Robinson and Oddy (2016) in that both MY and an alternative of methane production adjusted for liveweight (MPadjWt) were compared in a selection index. MPadjWt is a simpler, cost-effective measurement that does not require feed intake to be measured and also provides information on feed intake and efficiency as well methane emissions (through the correlations between these traits). When used in this way, profits increase and methane emissions are reduced even for a carbon price of zero. The three cost scenarios considered by Robinson and Oddy (2016) were **A:** a methane price of zero; **B**: $14/tonne $CO_2$-equivalent and methane's current 100-year Global Warming Parameter (GWP) of 34; and **C**: B: $30/tonne $CO_2$-equivalent and methane's current 20-year GWP of 88. These are equivalent to carbon prices of $19 and $105.6 on the scale (based on a GWP of 25 for methane) used by Fennessy et al. (2015),

In scenario A (no carbon price), one round of selection was estimated to increase profit by $20.69 per animal when methane is not measured. The return from increased slaughter weight of sale cattle in this scenario is offset by increased feed costs. The addition of 3 methane measurements using a Green Feed Emissions Monitor (GEM) was estimated to increase the profit per animal to $26.96, because of increased efficiency i.e. reduced feed intake and associated costs. There was an additional bonus of lower methane emissions than would be expected in the absence of methane measurements and a reduction in methane emissions per kg of saleable product.

As noted above MPadjWt is a simpler, more cost-effective measurement that does not require feed intake to be measured. Together with additional profits generated for breeder and producers, use of MPadjWt is therefore likely to increase the uptake of methane measurements compared with a selection system based on MY. Greater uptake is likely to increase the potential for real reductions in greenhouse gas emissions. MPadjWt is therefore recommended as a potential future trait in genetic evaluation selection systems.

A revised calculation (MACC analysis) of benefit of selection using alternates to MY such as $CH_4$ g/d or $CH_4$ adjusted for liveweight (g/d) needs to be conducted to ascertain the trade-off between productivity (profit) and mitigation potential of alternate means of expressing methane in a selection index. This is yet to be completed, but it is anticipated that the potentially low impact of selection through including methane traits, and low mitigation potential should be revised upwards. The MLA (2015) needs and gaps analysis conducted on the NLMP (National Livestock Methane Project) was not flattering to genetics, because it used selection on MY as the trait. As shown here, that leads to only small change in methane production and no change in productivity. This needs to be recalculated using the $CH_4$ trait as $CH_4$ adjLwt.

# 5. Future research needs

This research has clearly shown that methane production by sheep has a heritable component. The estimates of heritability for different ways of expressing methane traits are sufficiently promising to consider use by the sheep industry. There are additional industry benefits from measuring methane (estimation of feed intake) that until other methods are available are useful in their own right.

Ongoing research is needed to obtain data and calculate genetic parameters to increase the accuracy of the estimates. This can be achieved by industry using the PAC protocols developed here. Additional funding may be required for Sheep Genetics to compute updated genetic parameters, but the process is routine and should not be expensive.

We plan to combine all measurements of methane production in portable chambers (from 2010-2011, and the present study) and recompute genomic breeding values. These may be useful, but will need ongoing measurement of methane production by progeny of leading young industry sires to maintain currency, and long term industry value.

With respect to use of short term methane measurements as proxies for feed intake, we have recently developed a procedure using a 3-axis accelerometer to measure time spent grazing (Alvarenaga et al, 2016). Further work using this device in conjunction with portable chamber measurements of methane from progeny of leading industry animals would be worthwhile.

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# 7. Publications arising

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# 8. Appendices

## 8.1 Appendix – Support material for Robinson and Oddy (2016)

**Table A-1.** Plausible estimates of genetic (below diagonal) and **phenotypic (above) correlations**

| | SltWt | DMI | DMP | MPadjWt | MY | RFI |
|---|---|---|---|---|---|---|
| 1 SltWt | 1 | 0.59 | **0.47** | 0.00 | 0.03 | 0 |
| 2 DMI | 0.63 | 1 | 0.45 | 0.25 | 0.00 | 0.52 |
| 3 DMP | **0.64** | 0.84 | 1 | 0.40 | 0.20 | 0.24 |
| 4 MPadjWt | 0.00 | 0.57 | 0.77 | 1 | 0.15 | 0.28 |
| 5 MY | 0.00 | -0.04 | 0.30 | 0.35 | 1 | -0.08 |
| 6 RFI | -0.04 | 0.44 | 0.41 | 0.52 | -0.08 | 1 |

SltWt = weight at slaughter; DMP = daily methane production; MPadjWt = methane production adjusted for weight; MY = methane yield; RFI = Residual Feed Intake

**Sources and justifications of estimates of genetic (rg) and phenotypic (rp) correlations (numbered by rows in the above matrix)**

**Correlations with weight at slaughter (SltWt) DMI:**For cattle, weighted mean estimates from the 6 studies cited by Arthur and Herd (2008) and Crowley et al. (2010) are: rg = 0.72, rp = 0.61, for weight (during a feed efficiency test) with DMI measured over at least 50 days. Genetic correlations of weight with DMI in lambs are similar (rg = 0.85, François et al., 2007; rg = 0.71 ± 0.11, Snowder and Van Vleck, 2003, rg = 0.71, rp = 0.29, Lee et al., 2002) but lower for adults (rg = 0.34 ± 0.22, rp = 0.35 ± 0.03, Lee et al., 2002; rg = 0.20 ± 0.09, rp = 0.12 ± 0.03 for digestible DMI in Merino ewes, measured by chromium sesquioxide capsules and expressed as a ratio of the estimate for each ewe to the mean of the contemporary group, Fogarty et al., 2009; rg = 0.23 ± 0.10, rp = 0.15 ± 0.02 for correlations of same trait with post-weaning weight in crossbred ewes, Fogarty et al., 2006). In this evaluation, slaughter weight was considered a proxy for meat production, so correlations for non-mature animals were considered the most relevant. After 'bending' to make the correlation matrices positive definite, the values used in the evaluation were: **rg = 0.63, rp = 0.59**.

**SltWt, DMP:** The table below shows estimates of rg and rp from studies in Australia using RC and PAC. The RC protocol of restricted feeding based on a function of liveweight is likely to result in higher estimates of the correlation between weight and DMP than expected under commercial conditions when animals have ad lib access to feed. Therefore, the estimates from PAC of **rg = 0.67** and **rp = 0.47** were considered appropriate. After 'bending' to make the correlation matrices positive definite, a slightly lower value of **rg = 0.64** was used in the final analysis.

| rg | **rp** | **Data source for correlations of DMP and Wt (as an indicator of SltWt)** |
|---|---|---|
| 0.79 | **0.56** | Australian cattle, RC (Donoghue et al., 2015) |
| 0.67 | **0.49** | Australian sheep, PAC (unpublished result, from data analyzed by Robinson et al., 2014b) |
| | **0.45** | Australian sheep, PAC (unpublished result, data analyzed by Robinson et al., 2015) |

**SLtWt, MPadgWt:** rp should be 0 because of the adjustment for weight. There is no evidence that rg differs from 0, so the values used were: **rp = 0, rg = 0.**

**SLtWt, MY:** Genetic correlations correlations were low in studies that report them, e.g: rg = -0.10 ± 0.18 for test weight, 0.05 ± 0.17 for final weight in cattle (Donoghue et al., 2015) and 0.06 ± 0.12, 0.06 ± 0.13 for weaning and 8-month weight in sheep (Pinares-Patiño et al., 2013). Given the relatively large SE, the best estimate is **rg = 0.** Phenotypic correlations with liveweight had lower SE and were are all positive, rp = .04 ± 0.04 for test weight, rp = .10 ± .04 for final weight in cattle (Donoghue et al., 2015), rp = 0.01 ± 0.02, 0.03 ± 0.03 for weaning and 8-month weight in sheep (Pinares-Patiño et al., 2013). The pooled estimate (used in Table 5) is **rp = 0.03** ± 0.01.

**SLtWt, RFI:** rp should be 0 because RFI is a measure of feed intake adjusted for weight and weight gain. The weighted average of estimates from the studies cited in Arthur and Herd (2008) and Crowley et al. (2010) was very low: rg = -.06 ± 0.05. Although this is close to zero, the pooled value was considered preferable to simply rounding the average to zero because of the possibility that the genetic correlation differs according to environment. In one study (Morris et al., 2014), low-RFI heifers (progeny of 4 low-RFI sires, range –0.82 to -1.16 kg/day) had faster weight gains than the progeny of 4 high-RFI sires (range 1.0 to 1.14 kg/day), suggesting a possible negative correlation between RFI and weight. However, this particular study did not provide information on EBVs for 400-day or final weight, so the results could simply reflect the differences in sire EBVs for weight. After bending to ensure the correlation matrices were positive definite, a slightly lower value of **rg = -.04** was used (Table 5).

**2. Correlations with DMI**

**DMI, DMP:** DMP is highly correlated with DMI, but only a few studies report estimates of the correlation. For beef cattle, Donoghue et al. (2015) provided estimates of: rg = **0.84** ± 0.06, rp = 0.71 ± 0.02. The phenotypic correlation of 0.71 is for measurements in the same 2-day session. Based on an expected correlation of 0.54 between a single day's

measurements and the mean of 30 other measurements for DMI (using the repeatability estimate of 0.31 for feed intake of beef cattle on non-consecutive days, Robinson and Oddy, 2001), a plausible value for the phenotypic correlation between DMP and DMI average DMI over a period of at least 30 days is: **rp = 0.45.**

**DMI, MPadgWt:** The compiled estimates of the genetic correlations in Table 5 (discussed above) are 0.84 for DMI with DMP and 0.63 for DMI with weight. Assume the breeding value for DMI, bvi, can be decomposed into bvi = bviw + bvir, where bviw is the component associated with weight (accounting for 0.63*0.63= 40% of the variation) and bvir the remainder, which therefore accounts for 60% of the variation. If the genetic correlation of bvir (the proportion not associated with weight) and MPadjWt (the proportion of MP not associated with weight) is similar to the genetic correlation of DMI and DMP (0.84), a plausible estimate of the genetic correlation of DMI with MPadjWt is 0.84*sqrt(0.6) = 0.65, which was reduced to **rg = 0.57** to ensure a positive definite matrix (Table 5). This value is consistent with the expectation of a similar, but slightly lower genetic correlation between DMI and MPadjWt than between DMI and RFI (0.73, see below). The phenotypic correlation is expected to be substantially lower because MPadjWt is affected by feed intake on the day of measurements and previous two days, so subject to additional variability from day to day variation in feed intake. A plausible value is therefore **rp=0.25**.

**DMI, MY:** Not all studies report correlations for DMI and MY. Estimates from Donoghue et al. (2015) are: **rg = -0.04** , rp = -0.01± 0.04 for DMI over the period that MY was measured. The phenotypic correlation in Table 5 is for DMI measured over 30+ days, which is expected to have a lower correlation than for the period over which MY is measured, so the value rounded down to **rp = 0**.

**DMI, RFI:** Pooled estimates from the 6 studies cited by Arthur and Herd (2008) and Crowley et al. (2010), weighted by the variances of estimates (rg) or numbers of animals (rp) are: rg = **0.73; rp = 0.62**. RFI was included as the last row and column of Table 5, to provide an indication of the correlated response to selection. It has zero economic weight (so its inclusion should not affect the results for other traits). This last row was subject to a large amount of 'bending' to ensure positive definite matrices, after which the estimates were: **rg = 0.44**, **rp = 0.52.**

### 3. Correlations with DMP (not adjusted for weight or DMI) in normal production conditions

**DMP, MPadjWt:** The pooled estimate of rg for DMP and Wt (0.64, noted above) implies that weight explains 41% of the genetic variation, i.e. MP = $\beta$wt + e, where e (representing MPadjWt) has 59% of the variation. An estimate of the genetic correlation is therefore var(e)/sqrt(var(e)*var(MP)) = sqrt(var(e)/var(MP)) = sqrt(.59), i.e. **rg = 0.77**. When MP and MPadjWt are recorded on separate occasions, rp is expected to be much lower (because each is subject to different measurement errors), so a value of **rp = 0.40** was used.

**DMP, MY:** Estimated genetic correlations ranged from rg = 0.5 (Donoghue et al., 2015, for simultaneous RC measurements of DMP and MY in beef cattle to rg = 0.1, estimated from correlations between sire means (adjusted for fixed effects) of PAC measurements of sheep in Western Australia (with and without adjustment for liveweight) and MY measurements of offspring of the same sires in New South Wales (Dominik, personal communication). The

average value of these two values, **rg = 0.30** was chosen as the most plausible estimate based on currently available information.

For simultaneous measurements based on the same RC data, phenotypic correlations varied from rp = 0.68 (Donoghue et al., 2015) to rp = 0.23 for the dataset considered by Robinson et al. (2014b), in which the sheep the fed at 20 g/kg, so methane emissions were related to liveweight (r = 0.68) and strongly related to an index of feed intake in the RC and two previous days (**FII**, r = 0.84); in this dataset, DMP was more highly correlated (rp = 0.45) with MY calculated by dividing DMP by FII. The lowest estimate of the phenotypic correlation, an average of 0.10 was for MP measured in PAC and RC measurements of MP adjusted for feed intake in the RC and previous two days (calculated from the data discussed by Robinson et al., 2015). Phenotypic correlations based on simultaneous estimates from the same dataset are likely to over-estimate the true value, which is unlikely to be greater than the repeatability of DMP (0.27 in beef cattle), so a value of **rp = 0.20** was used, being less than the repeatability of DMP in beef cattle but higher than the phenotypic correlation of MY in the RC and DMP in PAC for sheep.

**DMP, RFI:** If A explains $r_1^2$ of the variation in B, and B explains $r_2^2$ of the variation in C, A might be expected to explain $r_1^2 * r_2^2$ of the variation in C, suggesting that a rough estimate of the correlation between A & C is $r_1 * r_2$. Hence, based on rg = 0.84 for MP and DMI (Table 5) and the pooled estimate (before 'bending') of rg = 0.73 for DMI and RFI (based on the 6 studies cited by Arthur and Herd, 2008 and estimates from Crowley et al., 2010), rg is estimated as 0.84*0.73 = 0.61. Similarly, using rp = 0.45 for MP and DMI (Table 5) and rp = 0.62 for DMI and RFI (pooled estimate above, before bending), rp is estimated as 0.45*0.62 = 0.28. Similar to the estimates for DMI, RFI, these estimates were subject to a large amount of 'bending' to ensure positive definite matrices, resulting in **rg = 0.41**, rp = 0.23 (used only to estimate correlated changes in RFI).

**4. Correlations with MPadjWt**

**MPadjWt, MY:** As described above, the pooled estimate of rg for DMP and MY was 0.30 (Table 5). The very low to zero correlations of MY with Wt suggest that adjusting MP for Wt is likely to increase the correlation, so a value of rg = **0.35** was used. Phenotypic correlations from sheep RC data were quite low (0.1 for MY0 and 0.19 for MY3) as was the average correlation of 0.1 for PAC MPadjWt and RC measurements of MP adjusted for feed intake (Robinson et al., 2015), so a value of **rp = 0.15,** between the lower and upper estimates was used for PAC MPadjWt and RCMY.

**MPadjWt, RFI:** a similar genetic correlation is expected to that of MP and DMI, or perhaps somewhat lower after accounting for weight adjustment in both variables. The estimate of 0.84 for MP with DMI was therefore reduced to 0.66, and further reduced by the bending procedure to rg = **0.53**. A relatively low value of rp = 0.3 was considered likely because of low repeatability of MPadjWt; this value further reduced by the bending procedure to **rp = 0.28.**

**5. Correlation with RCMY**

**MY, RFI:** some studies indicate that low-RFI (i.e. efficient) animals may have higher MY, e.g. Mercadante et al., (2015) tested 118 cattle for RFI during the growth phase then measured methane emissions on a subset of 23 males and 23 females; the low-RFI group had higher MY (25.1 *vs* 22.8, $P < 0.001$) than the high-RFI group. The 23 males underwent a second RFI

test over the same period as their methane emissions were measured and classified by the second RFI test as 9 low and 14 high RFI animals, for which there was no significant difference in MY (*P* = 0.38). In view of these results, the correlations were assumed to be low, but negative: **rg = -0.08, rp = -0.08**.

**Sensitivity testing.** Additional sensitivity analyses were conducted using higher estimates of correlations for DMP and MY. The estimate of rp was increased to 0.40 and **rg** to **0.47** (close to the reported value for simultaneous measurement in RC, based on a model that did not account for G x E effects that result in lower correlations for repeat methane measurements after 2 month interval [0.27 for DMP, 0.21 for MY] than on consecutive days [0.95 for DMP, 0.85 for MY, Donoghue et al., 2016]. To ensure the genetic covariance matrix remained positive definite, it was necessary to increase rg for MY with MPadjWt (to 0.57), reduce the correlation of DMP with DMP by 1 percentage point (to 0.83); a genetic correlation of zero was also assumed for DMI with MY.