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Improving production efficiency and reducing methane emissions in meat and wool sheep

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Abstract

Development of robust protocols to measure a methane phenotype on large numbers of animals is a first step to towards establishing the feasibility of reducing methane emissions by sheep selection. This is a progress report of a component of a larger project and specifically aimed to establish relationships between feed intake, proxies for feed intake and methane production from ewes fed different diets and across different physiological states. Methane production varied by almost 2-fold across diets but the effects of pregnancy status on methane production was relatively small. There was no significant interaction between sire and pregnancy status or reproductive rate at either site, which suggests that differences between sire groups was maintained across physiological states. Feed intake over the 24-48 hours prior to methane measurements explained significant variation in methane production in addition to that explained by live weight, particularly when sheep were fed a forage based diet under controlled conditions. Preliminary analysis suggests that production of carbon dioxide could be used as a proxy for feed intake in analysis of methane production. The estimates of the repeatability of methane production, with or without adjustment for live weight and or feed intake, and across a range of diets and or physiological states, were within the ranges reported elsewhere. The repeatability tended to be similar for two versus three measurements per animal or for methane tests over 40 or 60 minutes. Together with evidence that accumulation of carbon dioxide above 4% may increase the rate of methane production, it is hard to justify a test length using portable accumulation chambers longer than 40 minutes. Despite the repeatability of 0.2 to 0.4, depending on whether the data was adjusted for live weight or not, unlike the results from the NSW site there was a significant sire x diet interaction in the WA study. This may suggest that different genetics could be involved in methane production across different diets, but we currently have insufficient data to confirm this or derive genetic parameters.

Executive summary

Sheep and cattle produce 60-70% of agricultural greenhouse gas emissions in Australia, and these gases are predominantly methane from rumen fermentation. Reducing methane emissions from livestock is an emerging issue for agriculture and livestock production. Of the possible options to mitigate methane emissions exploiting the differences in methane production between individual animals through genetic selection appears to be the most likely strategy that could be adopted in extensive grazing systems. Development of robust protocols to measure a methane phenotype on large numbers of animals is a first step to towards establishing the feasibility of reducing methane emissions by genetic selection. This is a progress report that summarises the first phase of a larger project. The work reported here was undertaken in NSW and WA and specifically aimed to establish relationships between feed intake, proxies for feed intake and methane production from ewes fed different diets and across different physiological states.

In NSW methane production from about 100 ewes from four sires selected for extremes in methane production was measured several times using respiration chambers and or portable accumulation chambers. The measurements were completed when dry ewe were fed indoors a forage based diet either ad libitum or at maintenance or when they grazed different pastures, plus when fed ad-libitum and 1.6 x maintenance (calculated for dry ewes, approximately maintenance for pregnant ewes) indoors during late pregnancy. Where possible intakes were measured plus detailed measurements of oxygen and carbon dioxide concentrations. The ewes were CT scanned to characterise the reticulo-rumen complex and rumen fluid samples collected to quantify VFA's. In WA, almost 400 ewe lambs were measured for feed intake and methane production post-weaning when fed pellets ad libitum indoors. They were then mated as ewe lambs and methane production was again measured when they were grazing green pasture when the ewes were pregnant or dry.

Methane production varied by almost 2-fold across diets but the effects of pregnancy status on methane production was relatively small. At the NSW site there was a small negative effect of pregnancy status on methane production rate measured in respiration chambers after adjustment for feed intake and live weight. This effects was also evident at the WA site and methane production adjusted for live weight was 8% lower in pregnant than dry ewes. A reduction in methane production rate would be anticipated during pregnancy if there is a concomitant decrease in retention time of digesta in the rumen. The reduction in methane after adjustment for live weight could also reflect that the live weights used included the weight of the conceptus, which was probably about 10% of live weight given measurements were completed around day 125 of pregnancy. In any case, although there may be a reduction in methane production rate with pregnancy status, the magnitude of the effect was such that at present there is no case for adjusting the inventory to account for it. There was no significant interaction between sire and pregnancy status or reproductive rate at either site, which suggests that differences between sire groups was maintained across physiological states.

Feed intake accounted for up to 70% of the variation in methane emissions at the NSW site, but less than 30% at the WA site. The variation in methane production explained by intake was considerably lower at the WA site than was observed at the NSW site, probably reflecting real differences in diet composition and total intake but also greater errors in estimation of intake over short periods using the automated system in WA. The effect of feed intake on methane production was greatest in time

periods closest to measurement of methane. At the NSW site feed intake beyond 2 days prior to measurement had no significant effect on methane production, whereas at the WA site methane production was not related to intake beyond 1 day prior to measurement. Feed intake over the 24-48 hours prior to methane measurements explained significant variation in methane production in addition to that explained by live weight, particularly when sheep were fed a forage based diet under controlled conditions. Measurement of feed intake is currently not possible under grazing conditions, but preliminary analysis suggests that production of CO_2 could be used as a proxy for intake in analysis of methane production. CO_2 production rate and live weight accounted for approximately 63% of the variation in feed intake in the NSW study. CO_2 in particular can be measured at the same time as CH_4 and accounts for more variation in intake than live weight. There are also other reasons for measuring CO_2, we found that when CO_2 concentration in the portable chambers approached 4% at the end of the measurement period, that the rate of production of CH_4 increased. It is recommended that CO_2 be used to provide a quality control check on the CH_4 measurements within portable accumulation chambers.

There are theoretical reasons to believe that VFA production rate is in part a determinant of methane production, however there is no evidence in the current study that simply measuring VFA concentration can be used an indicator of methane production. This is similar to results reported elsewhere. There is a suggestion that there may be genetic correlation between methane production rate and the proportion of VFA as propionate in sheep and cattle, but as yet we have insufficient evidence to confirm those observations. There was also no sire effect on rumen volume after correction for live weight, and no significant difference between sire progeny groups in the proportion of different anatomical structures in the reticulo-rumen.

The estimates of the repeatability of methane production, with or without adjustment for live weight and or feed intake, and across a range of diets and or physiological states, were within the ranges reported elsewhere. The repeatability tended to be similar for two versus three measurements per animals or for methane tests over 40 and 60 minutes. Together with evidence that accumulation of carbon dioxide above 4% increases the rate of methane production, it is hard to justify a test length within portable accumulation chambers longer than 40 minutes. Despite the repeatability of 0.2 to 0.4, depending on whether the data was adjusted for live weight, unlike the results from the NSW site there was a significant sire x diet in the WA study. This may suggest that different genetics could be involved in methane production across different diets, but we have not yet sufficient data to add derive genetic parameters.

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1. Background

Context of this project

Animal agriculture produces 60-70% of agricultural greenhouse gas emissions in Australia, and these gases are predominantly methane from rumen fermentation. Reducing methane emissions from livestock is an emerging issue for agriculture, not just in Australia but in many countries. Of the possible options to mitigate methane emissions (reviewed by Buddle *et al*. 2011), exploiting the differences in methane production between individual animals through genetic selection appears to be the most likely strategy that could be adopted in extensive grazing systems. However, there are several issues that need to be overcome before this strategy could be implemented. One is to identify the practical aspects of a methane trait to include as part of a breeding objective. This could be methane production or methane production adjusted for live weight, feed intake and or some other associated parameter. The other issue is the cost and logistical complexity of measuring a methane phenotype on the large numbers of animals required to achieve this outcome. Selection for reduced methane emissions, if possible, should also be done in such a way as to not compromise other economically important production traits. In practice, it's likely that the processes used to measure methane will provide data and insights into feed intake, which is an integral part of efficiency of livestock production.

The project described here was conceived as part of an application to DAFF Filling the Research Gaps in 2012. It built on experience gained in attempts to generate genetic parameters for methane in the Sheep Information Nucleus Flock (B.CCH.1015). A major learning in that project was that much more attention on a measurement protocol and on repeat measures of each animal was required. It was also not clear the extent to which knowledge of feed intake would be required as part of a measurement procedure. The current project was an interim project to bridge a gap between B.CCH.1015 and support from DAFF, MLA and AWI to revisit measurement of industry animals to obtain enough quality data to resolve the issues identified as a result of B.CCH.1015. On-going funding from DAFF, MLA and AWI has now been achieved and we will continue the data collection and analysis described here under a new project "Genetics to reduce methane emissions from Australian sheep".

Technical considerations

Portable Accumulation Chambers (PACs) were developed for use in B.CCH.1015 to provide estimates of methane production quickly and relatively inexpensively (Goopy *et al*. 2011). We then used this technology to measure methane production and provide preliminary estimates of hereditability of the trait in over 3200 sheep in the field (see report to B.CCH.1015). The objectives and activities of this bridging project and the new project funded by DAFF, MLA and AWI are as follows:

a) Improve the methodologies for measurement of methane and methane yield, together with feed intake and efficiency in sheep. This will ultimately underpin a low cost rapid test of methane emissions and methane yield in the field (Recommendation 7 of Amer and Fennessy 2012);

b) Establish relationships between feed intake and methane production in different stages of a ewe's life (young growing, pregnant, lactating and mature non-pregnant non lactating);

c) Establish relationships between feed intake and methane production under different feeding, pasture and seasonal conditions;

d) Extend the data collected in BCCH1015 by more extensive phenotyping of current relatives. The purpose of this is to capitalise on the existing genotypes and phenotypes, and to improve the accuracy of the sire estimates from progeny which already have measurements of methane emissions;

e) Collect more extensive data on animals representative of the Australian sheep flock (Recommendation 9 of Amer and Fennessy 2012) to establish genetic parameters; and

f) Establish the potential for methane mitigation from indirect selection for indicator traits including improved feed efficiency or direct selection for lower methane production or lower methane yield and their likely impacts on farm profitability and adoptability

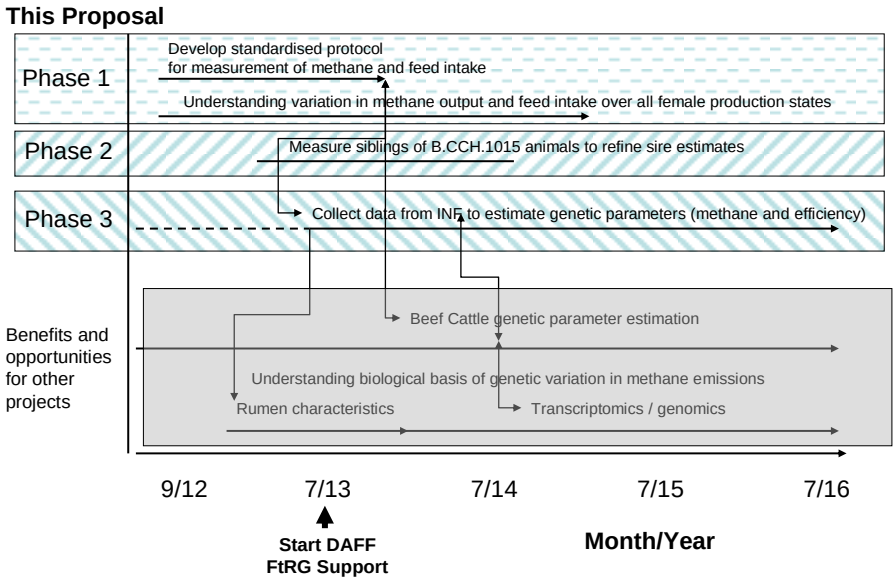
These activities logically fall into three phases of work, which are being addressed through co-ordinated sites in WA and NSW. These are:

1. Establish the relationships between feed intake and methane emissions over the normal production cycle of sheep and over a variety of pasture conditions, and from this develop a robust protocol for measurement of methane and feed intake (activities a, b and c above).

2. Using the best protocol, measure relatives of those animals already measured in past studies. This will maximise benefits from existing measurements on animals with good phenotypes that have already been genotyped (activity d above).

3. Measure new animals from the sheep information nucleus flocks at Kirby (UNE, NSW) and Katanning (DAFWA, WA) to generate sufficient data to permit estimates of genetic parameters to be made (activity e above).

There is a degree of overlap between the first and second phases of this work and it is imperative to move quickly to collect data for Phase 2 to not lose the opportunity to sample close relatives of the INF animals already measured in B.CCH.1015. A bulk of the work required for Phase 1 will be undertaken by the current bridging project, and the completion of Phase 2 and Phase 3 will be undertaken by the larger project funded by DAFF, MLA and AWI. A schematic of how the phases of this proposal fit together, and contribute to addressing more general questions about the sources of variation in efficiency and methane emissions in sheep and other species is shown in Figure 1 below.



The basis for each of the above activities is as follows.

a) Improved methodologies for measurement of methane and feed intake in sheep - Earlier work to develop a quick and comparatively inexpensive method to measure methane in lots of sheep lead to the development of the portable accumulation chamber (Goopy *et al*. 2011, B.CCH.1015). The development of a standard and reliable protocol for use has not yet been completed. Analysis of all suitable data in Australia and of similar techniques (short term measurements) in NZ indicate that repeatability of this method, after adjusting for liveweight as a proxy for feed intake, is approximately 0.2 to 0.3. Where feed intake data has been available, the evidence to data is that information on feed intake for at least the previous 3 days is required to remove variation in methane emissions due to variation in feed intake. However, this data has been collected where feed intake has been limited due to the protocol used, and certainly variation in feed intake was substantially less than would be observed if ad-libitum intake was allowed. The results from Australia (NSW and WA) and NZ are remarkably similar suggesting that several measurements of methane are needed (over a period of several months) and measures of feed intake leading up to methane measurement need to encompass at least 3 days. The period of measurement required for a stable estimate of feed intake is not well known in sheep, or whether, because of the high correlation between methane emissions and feed intake, the former could be used as a proxy for the latter. The patterns of feed intake in penned sheep are self-similar over different time scale (weeks and months) suggesting that at least several weeks of data is required. In cattle studies the error structure of feed intake for individual animals stabilises around 30-40 day (Archer et al, 1997). However, shorter time periods may be adequate if the aim is to evaluate methane emissions of sires. The error structure and appropriate periods for measuring feed intake need to be determined in sheep. Andrew Thompson has some longer term feed intake data collected in animals at Medina in WA which will be examined to inform the development of a robust methodology. At the recent (July 21, 2012) meeting of the Animal Selection Genetics and Genomics Network of the Global Research Alliance for reducing greenhouse gases from agriculture, this question was discussed at length. It is proposed that a working group of the ASGGN be established to help provide a common set of protocols for measurement of methane in international projects. The work proposed above will directly inform that working group (of which Dr Oddy is the Chair).

b) The relationship between intake (and methane production) of the same sheep during growth and at different stages of their productive life is not known. Many of the tests to estimate characteristics of feed intake and methane production, along with production traits, are conducted in young growing animals. Most of the feed eaten and methane produced in a flock is by adult ewes. There are a limited number of studies in which feed intake has been measured throughout the lifetime of sheep, and almost none where the same animals were growing, pregnant, lactating and dry. We will determine the magnitude of the phenotypic correlations between feed intake, growth rates, efficiency of wool growth and liveweight gain and methane production measured at different stages of an animal's life. There is an opportunity to generate this data from 2 sources derived within B.CCH.1015 and precursor projects. The first is from Merino ewes measured for feed intake and methane production as young growing animals that have subsequently lambed (Pingelly, WA) and the second is from Merino and Cross-bred ewes on which we have repeat measures of methane production, and their progeny from Merino sires whose progeny differed in methane production (Glen Innes / Armidale, NSW).

The following activities are part of the DAFF Filling the Research Gaps Round 2 project "Genetics to reduce methane emissions from Australian sheep", and are not part of the work to be conducted in this bridging project. They are included here only to provide context for the work above to be undetaken.

c) Extend data collection on relatives of those already measured in B.CCH.1015. Methane measurements were recorded on over 2500 sheep from the Sheep CRC information nucleus (INF), at least 1400 from the maternal efficiency flock (MEF) in WA and 700 from the former sheepGENOMICS flock in NSW. Only one measurement of methane production was made on each animal from the INF and different protocols were used. With the exception of 160 sheep of the former sheep GENOMICS flock in NSW and sheep from the MEF in WA, no data was collected on feed intake. New protocols have also recently been developed to substantially improve the accuracy of feed intake data collected from the facility recently set-up in WA. Almost all of the animals are genotyped, or could be genotyped in the case of the MEF, with the ovine SNP50 chip. The utility of these resources could be enhanced, if it were combined with data on methane production and feed intake on relatives, and if the data were measured by a common and well characterised protocol, including repeat measurements under different pasture conditions. We propose to select a subset of animals from the INF that are related to the animals for which we already have measures of methane production, and combine datasets to improve the accuracy of the estimate of sire variation in methane production and augment it with feed intake data. This will maximise the value of the data already collected in B.CCH.1015.

d) Measure new animals from the INF with a well described and calibrated protocol. This is likely to require multiple measures of methane production and at least one estimate of feed intake on each of the progeny. On a selected subset of progeny we will measure methane production in respiration chambers to strengthen confidence in methane estimation using alternate techniques. We anticipate measuring 1000 animals / year in both NSW and WA. The data obtained will inform the development of genetic parameters and because it will be measured over different seasons and pasture conditions on animals with good phenotypic data for production and product quality traits it will eventually contribute to estimates of genetic correlations between intake, methane and production and product quality traits including estimates of efficiency.

2. Project objectives

This project addresses a number of key research questions:

a) What is the best method of measurement (protocol for managing animals prior to and during data collection, and 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 measures taken at different times in an animal's life are correlated?

The outcomes from this project will be an accepted protocol for measurement of methane and feed intake in sheep for subsequent measurement of methane for establishing genetic parameters.This outcome is an essential step to providing the means to deliver methods for selection of more efficient, low methane emitting sheep.

3. Methodology

3.1. NSW study

Ewes (96), approximately 12 months old from 4 sires (19 to 29 progeny per sire) 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 there 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 Field Chambers (Goopy et al, 2011). Methane concentration was recorded 30 and 60 minutes after entering the chambers. Methane was measured using a FID analyser (MX100053 ENVCO Wellington New Zealand). Carbon dioxide 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 concentration was measured using a FoxBox (Sable Instruments, Nevada, UAS). 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 field chamber measurements we commenced measurement of methane in respiration chambers. Eight ewes were measured for 22 hrs each day. Intake was recorded in the chambers and prior to entering 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 field chamber measurements were repeated. The ewes were 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. Two pastures were used, each with different composition and total availability. Ewes were removed from pasture 60 minutes before being placed in the field chambers.

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 Respiration Chambers 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 for1 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.

Data presented below are summaries. Where statistical analyses have been carried out Minitab 14 was used. All values for gas transactions are adjusted to STP.

3.2. WA study

Merino weaners with full pedigree information and Australian Sheep Breeding Values for ASBVs for growth, carcass and wool traits were sourced from the Maternal Efficiency Flock at Pingelly (32^0 32'S, $117^0$05'E) and a research flock at Kojonup (33^0 83'S, $117^0$16'E). A summary of the 371 ewes used in each of the experiments is given in Table 1. The 371 ewes represented progeny from 22 sires with an average of 16.7 progeny per sire (range 1 and 50).

Table 1. Initial age and live weight of Merino ewe weaners from flocks used to measure feed use efficiency, daily methane production and methane yield.

	Age (days)	Live weight (kg)
Group 1 (n = 216)	147 (range 121 to 162)	33.3 (24.0 to 44.5)
Group 2 (n = 155)	176 (range 155 to 186)	30.1 (15.5 to 42.0)

The general procedures for measurement of feed intake, feed efficiency and methane production has been described previously (Final Report B.CCH.1015). The lambs were transferred from their flock of origin to a feedlot facility at the Medina Research Station (32^0 22'S, $115^0$81'E) about 10-days prior to commencement of feed intake and efficiency measurements. They were stocked at less than 100 lambs per

feedlot (60 m x 20 m) and each feedlot was fitted with a water trough, self-feeder and hay rack. During this 10-day introductory period the lambs were offered straw *ad libitum* plus increasing amounts of a commercial pellet (90% dry matter, 12.5 MJ metabolisable energy/kg dry matter and 16% crude protein) such that after 10-days the lambs were consuming pellets *ad libitum*. The lambs were then housed indoors for 55 days for Group 1 and 32 days for Group 2 in pens (3.3 x 7.5 m) at a maximum stocking density of 15 lambs per pen.

The 15 pens in the feed intake facility were all fitted with a water trough and automated feeding units capable of weighing feed intake to the nearest 10 g. Sheep were identified by electronic tags and the feeding units were fitted with electronic tag readers that identified individual sheep each time they fed. Only one sheep at a time could feed. The feeders were fitted with a load bar and scales, enabling the recording of total feed intake and the number of meals for each sheep per day. Feed intake was also calculated for 4 hour increments over the 72 hours prior to measuring methane production. During the test period the lambs were weighed twice a week and residual feed intake was calculated using the current Australian beef cattle model taken from Knott *et al.* (2008). Residual feed intake results are not provided in this progress report. All lambs had their fat and muscle at the C-site measured by ultrasound at the start and end of the feeding period.

Methane production from individual sheep was measured using 16 portable methane chambers (122cm x 122cm x 56cm). Methane production for each sheep was measured on three occasions during the last 2 to 3 weeks of the period of *ad libitum* feeding. The portable chambers were constructed to trap all exhaled and eructed gases during a one hour collection period and the development and full description of the chambers is provided by Goopy *et al*. (2011). Individual sheep were moved from their pens into a race and positioned below an individual chamber that was suspended above the raceway. The chamber was then lowered over the sheep and secured. This process was undertaken within 15 minutes of the animal being removed from access to feed. A thermometer mounted in each chamber recorded start and end temperature for each animal. Methane concentration in the chambers was measured 20, 30, 40 and 60 minutes after the chamber was secured. This was done using a MicroFID flame ionization detector fitted with a 20 cm flexible silicon sampling tube introduced to each chamber through a sampling port. After the final measurement sheep were returned to their pens in the feed intake facility. Total gas space inside the portable chamber (*i.e.*, net volume) was estimated by assuming that the volume occupied by the sheep was equal to 1 L/kg liveweight and subtracting the liveweight of the sheep from the internal volume of the chamber. The estimated production in the chamber was then converted to estimated production over 24 hours. This was then corrected for volume at standard temperature and pressure (STP) using the following formula:

$$\text{STP correction} = \frac{273.1}{(273.1 + \text{temperature (°C)})} * \frac{\text{pressure (kPa)}}{101.3}$$

After completion of the feed intake and methane measurements at Medina, all ewes were transported to the research farm at Ridgefield and stocked on pasture. The ewes were then split into 3 groups and syndicate mated between February and April for 5 weeks after teasing, with the three groups mated 17-days apart (Pasture A, joined on 27-Feb; Pasture B joined on 16-March and Pasture C joined on 4-April) They were pregnancy scanned on 3-June. After mating the ewes were run as one mob for the remaining duration of the trial except during the periods when methane was measured. All animals were remeasured for methane between June and August when the pregnant ewes were between day 110 and 130 of gestation. Methane was

measured 3 times with about one week between each measurement. The dates for the methane measurements while the animals were in the indoor facility and grazing are shown in Table 2.

Table 2. Dates for measurement of methane for indoor and paddock groups in 2013. Days from the start of joining are shown in brackets for the three pasture groups.

		Measurement 1	Measurement 2	Measurement 3
Indoor	Group 1	9th & 10th Jan	16th & 17th Jan	29th & 30th Jan
	Group 2	28th Feb & 1st Mar	5th & 6th Mar	12th & 13th Mar
Paddock	Group A	26th & 27th Jun (119)	2nd & 3rd Jul (125)	8th & 9th Jul (131)
	Group B	12th & 13th Jul (118)	18th & 19th Jul (124)	24th & 25th Jul (130)
	Group C	2nd Aug (120)	8th Aug (126)	14th Aug (132)

Three days prior to methane measurements, ewes to be measured were drafted from the larger flock of ewe lambs and moved into a holding paddock which was managed to have similar levels of pasture for all groups. The holding paddock was further split into two areas of varying size and both areas had access to water. When measurements were completed over two days, on the first day of measurement the ewes were mustered into the yards, and then drafted into two groups. One group was measured that day and the second group was returned to the paddock to be measured the next day. The group to be tested on the first day was then drafted into groups of 15 and held in the sheep yards until their methane production was measured. Whilst in the sheep yards they did not have access to feed or water. If there were more than four methane runs completed in a day, only the first 4 runs remained in the yards, and the other ewes were returned to a smaller holding paddock and grazed as per normal. The maximum time sheep were off-pasture prior to methane measurements was 4 hours; time of pasture was recorded but is not included in the analysis provided in this report. After methane measurements were completed, each group of 15 ewes were moved to the larger holding paddock with adequate pasture and water for the night. For day 2 of measurements, the unmeasured ewes will be mustered, yarded and drafted similarly as described above. Once these ewes are out of the paddock, the ewes measured on day 1 were returned to the main pasture paddock. The ewes measured on day 2 were also returned to this paddock immediately after measurement.

Restricted maximum likelihood method (REML) was used to fit the various methane analyses with ewe source, age of dam, pregnancy status, methane group and live weight and interactions thereof, where appropriate, as fixed effects. Sire and interaction with methane group, where appropriate, along with animal and methane measurement date and run within measurement date fitted as random effects. For all analyses, terms were included only if they were statistically significant ($P < 0.05$). Repeatability was calculated from the estimated variance components derived using REML analysis. All statistical analyses were performed using GenStat (VSN International 2012).

4. Results

4.1. NSW – growing ewes

4.1.1. Sire and treatment summary

Summary statistics for growing ewes at the NSW site are shown in Tables 3 and 4

Table 3. Number of progeny per sire, live weight (kg) and average ad-libitum feed intake (g/d) over 5 days before measurement of methane for 1 hour. Values shown are means ± SD.

Sire	No of Progeny	Liveweight	Feed intake
1	29	54.6 ± 7.18	1691 ± 282.6
2	26	46.5 ± 6.22	1173 ± 242.6
3	22	52.2 ± 6.71	1500 ± 314.9
4	19	47.6 ± 8.08	1370 ± 356.7

Table 4. Summary statistics for rate of feed intake (g/min, averaged over 30-48 hours before measurement), methane emissions (ml/min), CO2 production (ml/min) and O2 consumption (ml/min). Values shown are means ± se.

Measurement period	Rate of feed intake§	CH4 (ml/min)	CO2 (ml/min)	O2 (ml/min)
Ad-lib 1	1.11 ± 0.034	25.9 ± 0.81	379 ± 8.2	-369 ± 32.3*
Ad-lib 2	1.01 ± 0.030	24.5 ± 0.80	347 ± 7.4	-326 ± 5.4
Respiration Chamber ***	0.89 ± 0.019	20.5 ± 0.45	317 ± 5.9	NA**
Maintenance 1	0.90 ± 0.001	23.0 ± 0.48	318 ± 6.1	-291 ± 4.6
Maintenance 2	0.92 ± 0.001	24.2 ± 0.51	308 ± 5.3	-292 ± 4.3

§ = intake calculated as rate (g/min) using appropriate period prior to measurement. In PACs = intake from day before + that eaten on day prior to measurement, in respiration chamber = intake from day before and day of measurement in chamber.
* n = 8 measurements
** NA = data not available
*** measured over 22hours calculated as average ml/min, PAC data calculated as average ml/min from 60 min measurement

4.1.2. Relationship between feed intake and methane emissions

The extent to which feed intake affected methane emissions was determined using data from all animals during the ad-libitum intake period. The contribution of measured feed intake for various periods of time before measurement of methane emissions (ml/min) over the 1 hour period in the PAC was determined by regression analysis. Table 5 shows that the effect of intake on methane production was greatest in time periods closest to measurement of methane (on day of measurement and the day prior) and had no significant effect beyond 2 days prior to measurement. Feed intake accounted for more than 70% of the variation in methane emissions.

Table 5. Relationship between methane emissions (ml/min) measured in PAC and feed intake during different periods prior to measurement of methane. PAC1 and PAC2 are measurements taken 2 days apart.

	PAC1		PAC2	
	t =	p =	t =	p =
Intake 4 d prior	0.55	0.582	0.9	0.37
Intake 3 d prior	-0.26	0.797	-0.59	0.56
Intake 2 d prior	-0.37	0.712	-0.88	0.38
Intake d before	2.6	0.011	4.08	0.000
Intake on day	9.31	0.000	9.11	0.000
% variation in CH4 emissions accounted for by intake	74%		78%	

4.1.3. Relationships between feed intake, live weight and methane emissions

In the absence of feed intake measurements, live weight has been used to adjust methane emissions in part to account for the obvious relationship between feed intake and methane emissions (Robinson *et al*. 2010) and in part to account for anticipated relationships between feed intake and live weight. Table 6 demonstrates that although there is a positive relationship between live weight and methane, much more variation in methane emissions is due to feed intake during the period immediately preceding the measurements of methane production rather than to live weight.

Table 6. Comparative AOV of relationships between feed intake, live weight and sire with methane emissions. Model fitted was methane (ml/min) vs sire with intake on day of measurement and day before measurement and / or live weight fitted as covariate(s). Example shown for PAC1 measurement.

Source	F*	F	F
Intake d before	11.4	31.3	
Intake on day	82.1	91.7	
Wt	6.72		38.7
Sire	2.53	3.24	3.98
df (error)	87	88	89
R^2 (adj)	74.3	72.6	45.8

There was a significant relationship between live weight and intake (accounting for approximately 45% of the variance) and this differed between sire progeny groups. Together these results suggest (not surprisingly) that feed intake and live weight are related and that the relationship varied between sire progeny groups. These results also suggests that although live weight has been used as a covariate to standardise methane emissions between individual animals, the association is driven by generally positive relationships between live weight and feed intake, but this association is not as good as a direct measure of intake.

Together this suggests that measurement of feed intake together with methane emissions is preferable to using live weight (if the objective is to understand the phenotypic relationships). The data indicate that intake for the period 24-36 hours before measurement of methane in a PAC is significantly associated with methane emissions and that information on intake periods beyond that have no statistical effect. These data suggest that the period over which feed intake should be measured prior to (and including) measurement of methane emissions over 1 hour should be approximately 36 hours.

To demonstrate how feed intake data can be used, methane yield was calculated from methane (ml/min) and rate of intake (g/min) and also from adjusted methane (ml/min) using rate of eating for 36 hours prior to measurement (Table 7). Methane yield was calculated for all measures (combined PACs and Respiration Chambers) after removing those measures where animal refused to eat more than 50% of intake on the day of measurement. Period effects were as expected in that methane yield was greater at maintenance intake than ad libitum intake, irrespective of the method used to calculate methane yield.

Table 7. Mean values (± se) of methane yield and methane output adjusted for feed intake by sire. Data are from two measures of 1-hour at ad-lib intake, 1 measurement in respiration chambers at ad-lib intake and two by 1-hour measures in PACs at maintenance intake. Rate of eating (g/min) was calculated from feed intake during the day before measurement + feed intake on the day of measurement / time.

Sire	MY (calculated as ratio)	CH4 adjusted for rate of eating
1	24.8 ± 0.28	24.4 ± 0.31
2	24.0 ± 0.30	22.9 ± 0.32
3	24.4 ± 0.33	23.8 ± 0.34
4	24.3 ± 0.35	23.3 ± 0.36
Adj R^2	11.3	69.8
Effect of sire (F 3,450)	1.18	3.39

4.1.4. Alternative measurements of feed intake

Accurate measurement of individual feed intake by large numbers of sheep at pasture over such a time scale has not been reported. However, if it were possible to find indicators of feed intake, other than or in addition to live weight, suitable adjustments for short term measures of methane emissions could in theory at least be constructed. There is a strong body of evidence linking energy expenditure of animals with weight and feed intake (embodied in practical terms in various feeding systems – NRC, SCA, AFRC etc). It is feasible to measure not only CH4 but also CO2 and O2 in accumulation chambers (and open circuit respiration chambers). If there are strong correlations between variation in feed intake with variation CO2 output and O2 uptake, it may be possible to use these gases as a proxy for feed intake under grazing conditions.

Carbon dioxide is produced during substrate oxidation by the host, and by anaerobic fermentation of feed (by microbes) in the rumen. In the host oxygen is consumed to generate energy for maintenance and growth. Oxygen consumption is directly related to heat production. When an animal ingests feed, there is an additional increase in oxygen consumption associated with short term changes in ingestion and digestion of feed and metabolism (Webster 1970). The heat increment of feeding and therefore oxygen consumption and CO2 output are directly related to feed intake. In energy terms heat increment of feeding is approximately 9% of metabolisable energy Intake (Corbett and Graham, 1980's, SCA, 1990). There are two sources of CO2, one from host metabolism, which should be directly proportional to O2 uptake, the other from fermentation of feed in the rumen. Relative proportions of CO2 from rumen fermentation compared with that from the host are from 10-20% depending on amount of feed ingested.

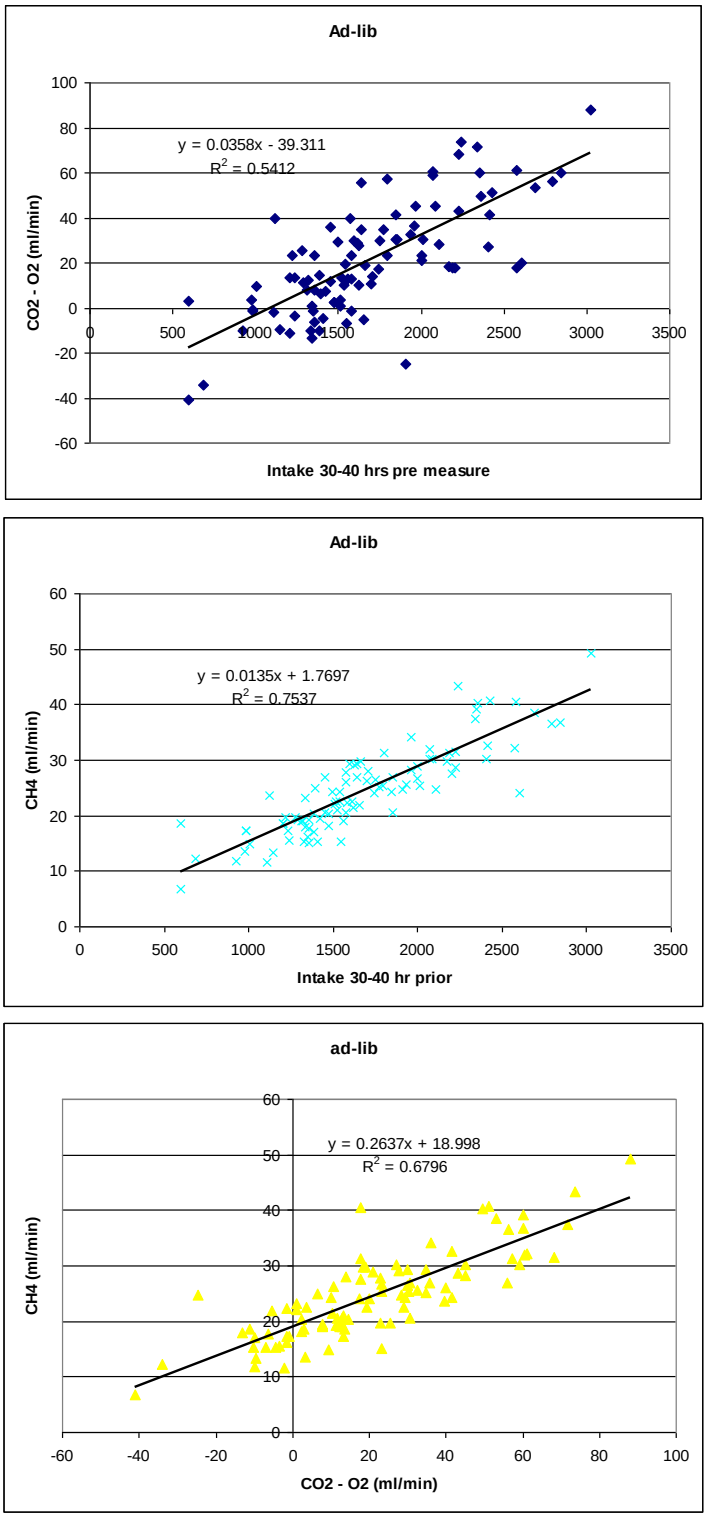


Figure 2. Relationships between feed intake, carbon dioxide production and methane production for sheep fed ad libitum.

The ranking of sires for methane yield calculated using the residuals from the general relationships shown in Figure 2 are given in Table 8. The absolute values of methane yield are higher because PAC1 and RC data did not have oxygen data (note these data refer only to PAC2, 3, 4 where oxygen was measured).

Table 8. Mean values (± se) of methane yield calculated using the residuals of the relationships shown in Figure 2.

Sire	Residuals CH4 fitted against intake	Residuals CH4 fitted against CO2 – O2	Methane yield (ml CH4 /g feed)
1	0.71 ± 4.3	1.45 ± 5.13	26.6
2	-0.96 ± 2.8	-2.06 ± 3.49	24.9
3	1.19 ± 4.7	0.68 ± 3.82	25.9
4	-1.14 ± 2.8	-0.19 ± 4.25	26.1

Calculation of methane yield is fraught with assumption of which intake to use. In this analysis feed intake during day before and on day of measurement up to time in PAC was used to calculate rate of eating. There was a significant period effect on methane yield; ad-libitum = 24.9 ± 0.41 was less than ($P<0.01$) than at maintenance 25.9 and 26.81 ± 0.41 (se). If anything the ranking of CH4 vs CO2 – O2 showed a closer fit to the ranking of methane than methane fitted against intake. Because oxygen data was not available for PAC1 and RC, feed intake and CO2 data for all measurements below was used after removing those measures where animals refused to eat more than 50% of intake on the day of measurement. Period effects were as expected (vis, methane yield was greater at maintenance intake, irrespective of method of calculating it.

Sire	MY (calculated as ratio)	CH4 adjusted for rate of eating	CH4 adjusted for CO2
1	24.8 ± 0.28	24.4 ± 0.31	24.8 ± 0.24
2	24.0 ± 0.30	22.9 ± 0.32	23.2 ± 0.30
3	24.4 ± 0.33	23.8 ± 0.34	22.2 ± 0.33
4	24.3 ±0.35	23.3 ± 0.36	23.9 ± 0.35
Adj R^2	11.3	69.8	81.6
Effect of sire (F 3,450)	1.18	3.39	20.77

The analyses below are for all the data from the Nov - Dec measurements. Feed intake during the day before and on the day of the measurement was used to calculate intake rate (g/min) as the denominator with CH4 (ml/min) as the top part of the ratio. For estimating methane yield using indirect measures of intake, regression techniques on things which are correlated (both statistically and logically through biochemical pathways) was used. Accordingly it is difficult to bring “untainted” = independent insights into this space. This is not helped by having insufficient data on contributing factors (wide range of intakes, measurement of O2 and CO2 along with CH4). Odd things are happening when working with these ratios. For example, when CH4/CO2 was used as an index of CH4/FI = methane yield, there should have in principal been a strong correlation between the two (CH4 -> FI R^2 >0.7, FI > CO2 R^ >0.7), but this was not the case (as below). Analysis and interpretation of this data is still in progress.

4.1.5. Summary of all measurement periods

Table 9. Summary of live weight (kg), methane, carbon dioxide emissions and oxygen uptake (ml/min) ± SD for 96 ewes, by period of measurement

Period	Wt (kg)	CH_4 (ml/min)	CO_2 (ml/min)	O_2 (ml/min)
1	50.5 ± 7.73	25.9 ± 7.82	379 ± 80.3	-369 ± 91.3*
2	50.5 ± 7.73	24.5 ± 7.80	347 ± 72.6	-326 ± 53.4
3	50.0 ± 7.54	23.0 ± 4.71	318 ± 59.4	-291 ± 45.6
4	50.0 ± 7.54	24.2 ± 5.02	308 ± 52.1	-293 ± 42.1
5	50.5 ± 7.73	20.5 ± 4.38	317 ± 57.5	*
6	52.4 ± 8.10	20.1 ± 5.36	430 ± 73.0	-451 ± 67.4
7	53.2 ± 8.31	20.8 ± 4.97	392 ± 54.3	-418 ± 50.8
8	53.5 ± 8.63	25.5 ± 6.13	381 ± 54.3	-417 ± 56.9
9	54.0 ± 8.32	24.9 ± 5.21	389 ± 60.0	-404 ± 52.7

* only 4 animals had O_2 measurements in Period 1.
Period 1 and 2 = ad-lib feed intake animal house, 1 hr PAC measurement
Period 3 and 4 = maintenance feed intake, animal house, 1 hr PAC measurement
Period 5 = ad-lib feed intake, respiration chamber
Period 6 and 7 = pasture ~1450 kgDM/ha, 1 hr PAC measurement
Period 8 and 9 = pasture ~ 1800 kgDM/ha, 1hr PAC measurement

4.1.6. VFA concentration and molar %

The molar concentrations of acetate, propionate, butyrate and valerate were significantly (P<0.001) and positively correlated with feed intake, whereas the molar concentration of iso-butyrate and iso-valerate tended (P = 0.07, iC4, P=0.01) to be negatively correlated with feed intake. Feed intake was therefore used as a covariate in subsequent analyses. In addition (i.e. in the same model) live weight was significantly negatively correlated with molar concentrations of C2, C3, C4 and C5 and not with iC4 and iC5, so live weight was used also as a covariate in subsequent analysis. Despite these adjustments, period remained significant. It was used together with a sire * period interaction to test if sire was significant for VFA concentrations and VFA molar %.

Table 10. Mean (± SE mean) and Analysis of Variance of VFA concentrations. Feed Intake the day immediately preceding the rumen sample and liveweight were fitted at covariates. Period, Sire and Sire* Period (S*P) were fitted as fixed effects in a General Linear Model trait~covars + P + S + S*P. Values shown below are F values, and P for each trait. F has 1, 182 df for all except Sire and S*P where has 3,182 df.

VFA	Mean (mM) ± SE	Feed Intake	Weight	Period	Sire	S * P	AdjR^2
Acetate	32.2±0.6	30.2***	13.7***	12.1***	1.2 NS	0.1 NS	60.3
Propionate	8.3±0.2	41.8***	18.4***	7.2**	0.3 NS	0.2 NS	61.1
i-Butyrate	0.7±0.01	3.9*	0.8 NS	39.6***	2.2 NS	1 NS	56.4
Butyrate	3.4±0.1	16.7***	4.3*	6.9**	2.6+	1.4 NS	43.7
i-Valerate	1.2±0.02	4.7*	3.6 +	9.7**	3.9*	1.7 NS	36.0
Valerate	0.4±0.01	15.1***	7.2**	2 NS	1.5 NS	0.5 NS	37.1
Total mM	46.1±0.9	33.3***	14.2***	10.1***	0.4 NS	0.1 NS	60.0

*** P<0.001, ** P<0.01, * P<0.05, + P<0.1 NS = P>0.1

Table 11. Mean (± SE mean) and analysis of variance of VFA Molar %. Same model used as for concentrations (above).

VFA	Mean (%)	Feed Intake	Weight	Period	Sire	S * P	AdjR^2
Acetate	70±0.15	6.3*	1.9 NS	0.8 NS	6***	0.3 NS	10.2
Propionate	17.7±0.14	24.6***	12.5***	1.6 NS	0.6 NS	0.8 NS	41.3
i-Butyrate	1.6±0.05	25.1***	8.5**	91.6***	3*	2.5 +	79.9
Butyrate	7.1±0.1	2 NS	0.2 NS	4.7*	45.3***	1.4 NS	24.3
i-Valerate	2.8±0.07	35.2***	18.6***	52.9***	3.2 *	2.5 +	76.5
Valerate	0.8±0.1	0.1 NS	0 NS	3.8 +	1.8 NS	2 NS	7.7
(C2+C4)/C3	4.41±0.04	20***	10.6***	0.1 NS	0.5 NS	0.6 NS	29.5

*** P<0.001, ** P<0.01, * P<0.05, + P<0.1 NS = P>0.1

The relationship between CH_4 and VFA concentration and Molar % VFA on ad-lib and maintenance intake was examined. Despite there being sire effects on Molar % C2 and C4 and differences between sires in CH_4 output, there were no significant relationships between CH4 output and concentration of total or individual VFA and / or molar %. There was no relationship between CH4 and (C2+C4)/C3 after adjusted for intake, weight rumen volume and sire. This suggests that VFA will not be a useful (phenotypic) predictor of CH_4.

4.1.7 Rumen volume and structure

Rumen volume was calculated from CT scan images of sheep when eating ad-lib and maintenance diets. Mean volume by intake period are shown in Table 12 below. There were significant differences between sires (P<0.01) and ad-lib and maintenance intake (P<0.01) (but no interaction between sire and intake period). Analysis that included feed intake the day prior to the CT Scan and live weight in the model removed the effect of sire. Weight of the animal was more strongly related to rumen volume than feed intake. This suggests simply that heavier animals have larger rumens. After adjustment for live weight, there were no significant differences in rumen volume due to sire. There were no significant differences between sire in the proportions of components of the reticulo-rumen complex, although there was trend (P=0.07) sire M5 to have a greater proportion of RR volume as Ventral Sac.

Table 12. Rumen Volume (mean ± SE mean, ml) by sire and period (unadjusted for weight or feed intake).

Sire	Ad-lib	Maintenance
M4	9657 ± 456.1	10643 ± 439.5
M5	7869 ± 456.1	8549 ± 456.1
MU1	9651 ± 507.5	10134 ± 507.5
W1	8680 ± 548.1	9203 ± 533.5

Table 13. Reticulo-rumen volume (cm^3), adjusted for weight, and proportions of component volumes by sire from ad-libitum intake period. Values are mean ± sem.

Sire	Reticulo-rumen Volume	Proportion of total		
		Reticulum	Dorsal Sac	Ventral Sac
M4	8980 ± 342.4	0.102 ± 0.0049	0.438 ± 0.012	0.460 ± 0.012
M5	8576 ± 343.2	0.091 ± 0.0049	0.423 ± 0.012	0.486 ± 0.012
MU1	9244 ± 370.2	0.099 ± 0.0053	0.447 ± 0.013	0.454 ± 0.013
W1	8940 ± 400.0	0.100 ± 0.0057	0.461 ± 0.014	0.440 ± 0.014

The relationships between rumen volume and methane production was explored. The analyses indicate that:

a) There was a significant positive relationship between methane production and rumen volume. It is not entirely clear if this is just because bigger sheep eat more, or because bigger sheep have bigger rumens. The analysis suggests it's the latter.

b) There is a difference between sires in unadjusted methane production (ml/min), and without including any of intake, weight or rumen volume, there is a period by sire interaction.

c) When you include intake, weight or rumen volume, there is a stronger sire effect and the sire * period interaction goes away.

d) Most of the difference in methane production is due to live weight and rumen volume, intake accounts for just a small additional component (p=0.055). However, this is in a data set where half the data were derived from animals fed in proportion to live weight. It would be dangerous to conclude that intake was not a contributor to methane production.

Overall, these results indicate that of the systematic variation in methane production between sires and some of this variation in methane production is accounted for by variation in rumen volume.

4.2. NSW pregnant ewes

4.2.1. Pregnancy status effects on methane production

The results from pregnancy scanning on June 25, 2013 are shown in Table 14.

Table 14. Summary of pregnancy scanning and proportion of ewes scanned with zero, single or twin foetuses.

Sire	n	singles	twins	empty
M4	29	15	10	4
M5	27	13	7	7
MU1	22	11	6	5
W1	20	11	5	4

The measurement of CH_4 production rate in portable accumulation chambers was made prior to (1.6*M) and after (ad-lib) measurements in respiration chambers. It is striking that rates of production of CH_4 are significantly higher in PACs than respiration chambers. This is because the rate of eating in the short period prior to measurement in PACs was typically more than twice the average rate of feed ingestion in the Respiration Chambers. The ewes ate a substantially greater proportion of their daily feed (at both 1.6 * M and ad-lib) before the PAC measurement whereas in the respiration chambers they ate more, but over the entire 22 hour period. Because CH_4 production is a rate, and it is responsive to short term inputs into the rumen, CH_4 production rate measured in the PACs reflects the actual intake immediately before the measurement.

Table 15. Mean values for CH_4 production rate (ml/min) in respiration chambers adjusted for the covariates live weight, feed Intake on day of measurement, and day previously, for progeny of 4 sires, at different pregnancy status (dry, single, twin). Numbers of progeny / sire are shown in the Table above. Ewes were measured when approximately 125 days pregnant. Feed offered was a mix of lucerne and oaten chaff offered at 1.6 * maintenance calculated on the basis of live weight. Measurements were made over 22 hours and intake recorded on day prior to measurement and while in the chambers.

	Overall	Pregnancy status		
Sire	Mean	0	1	2
M4	21.7	22.6	21.9	20.8
M5	21.3	22.4	21.5	20
MU1	21.5	22.8	22.1	19.6
W1	22	23.1	21.5	21.3

Average std error of mean for observations = 0.4 for sire mean and pregnancy status and = 0.8 for progeny groups in each sire * pregnancy status group

Analysis of variance. Significance of effect of

Liveweight	P=0.003
Feed Intake on day	P<0.001
Feed Intake day before	P=0.25
Sire	P = 0.63
Pregnancy Status	P = 0.002
Sire * Pregnancy Status	P = 0.73
	Adjusted R^2 = 72%

Table 16. Mean values for CH_4 production rate (ml/min) in portable accumulation chambers adjusted for the covariates live weight, feed intake on day of measurement, and day previously, for progeny of 4 sires, at different pregnancy status (dry, single, twin), at 2 levels of feed intake and stage of pregnancy. Numbers of progeny / sire are shown in the Table above. Feed offered was a mix of lucerne and oaten chaff offered at 1.6 * Maintenance calculated on basis of live weight at an average of 115 days post coitus (pc) and ad-libitum at 130days pc, Measurements were made over 60 minutes in portable accumulation chambers when fed 1.6 * M and 40 minutes when fed ad-libitum. Intake was recorded on day prior to measurement and up until the ewes entered the PACs.

	Overall	Pregnancy Status		
Sire	mean	0	1	2
M4	39.5	39.4	40.3	38.7
M5	39	38.9	39.1	39
MU1	40.2	40.4	38.6	41.5
W1	38.9	41.2	37.5	37.9

Average std error of mean for observations = 0.7 for sire mean and pregnancy status and = 1.2 for progeny groups in each sire * pregnancy status group

Analysis of variance

Liveweight	P = 0.001
Feed Intake on Day	P = 0.001
Feed Intake day prior	P = 0.001
Sire	P = 0.54
Pregnancy status	P = 0.43
Treatment	P = 0.5
Sire * Pregnancy status	P = 0.19
	Adj R^2 = 59.5%

An important result above is the observation that there was no sire* pregnancy status interaction, in either the respiration chambers or the PACs (which included 2 levels of feed intake). This suggests that if differences between sires are observed they are likely to be maintained across physiological state and intake level. Of course this will need confirmation in studies with a larger number of sires of markedly different intrinsic methane production rates. In fact the data show there was no significant difference in methane production rates between sire progeny groups during pregnancy after adjustment for feed intake and live weight.

4.2.2. Repeatability of different ways of expressing methane production

To estimate long term repeatability of different ways of expressing methane production, analyses of all PAC data using feed intake on the day and the day prior, live weight and CO_2 as a proxy for intake were conducted across measurements when ewes were growing and pregnant. The data with feed intake only was from growing ewes measured in Armidale in November / December 2012 and the same ewes while pregnant measured in July / August 2013. The data for live weight and CO_2 included the above, plus the pasture measurements at Glen Innes from February / March 2013 before the ewes were joined. The model fitted was: CH_4 production rate ~ Constant + Sire + Treat + Treat. Pregnancy Status + trait shown in the table below. Repeatability was calculated from the estimated variance components derived using REML analysis using Genstat v12.

Table 17. Estimates of repeatability of measurement of methane production rate (ml/min) using short term (40 min to 1hr) measurements in portable accumulation chambers.

Trait	Repeatability (4 periods, 2 growing, 2 pregnant, intake known)	Repeatability (6 periods, 4 growing, 2 pregnant, intake not known in 2 growing periods)
CH_4 alone	0.46	0.44
CH_4 adjusted for intake on day of measurement	0.30	-
CH_4 adjusted for intake on day of measurement and day before	0.17	-
CH_4 adjusted for Live weight and intake on day and day before measurement	0.12	-
CH_4 adjusted for live weight	-	0.21
CH_4 adjusted for CO_2 production rate	-	0.20
CH_4 adjusted for live weight and CO_2 production rate	-	0.18

The above results are similar to those reported by Pinares-Patino *et al.* (2013) and for CH4 adjusted for live weight by Robinson *et al.* (2012 – Final report B.CCH.1015). They suggest that multiple measurements of methane production rate are required to adequately characterise each animal. From experience, it is likely that genetic correlations within animals across different time points of measurement will be higher than indicated by the repeatability estimates. But, to compute genetic correlations between time points within animals will require considerably more data than currently available. The ranking of sire progeny groups across time and pregnancy status in the current data set suggest that the genetic correlations may well be higher than indicated by the repeatability. Nonetheless, it would be prudent to obtain multiple measurements on the same animals over time and across physiological state to generate the data sets required to estimate the required genetic correlations. A

simple rule of thumb would be that at least 2 and more likely 3 measures / animal will be required. The observation that repeatability of CH_4 production rate reduces as more adjustments for factors accounting for variation in CH4 production rate are included in the analysis is expected. It indicates that factors such as live weight, feed intake on day and day prior to measurement and CO_2 production rate account for substantial variation in CH_4 production rate. It suggests that in practice at least some of these covariates should be considered when forming a methane production trait.

4.2.3. Observations on measurement protocol in PACs.

Methane concentration was routinely measured at 2 times while the sheep were in the PACs. The percentage of CO_2 and O_2 was also measured and this data is used to calculate rate of gas exchange. During measurements of pregnant ewes (approx 115 d pc) eating lucerne / oaten chaff ad-libitum resulted in a number of recordings of CO_2 % were in the range 4-6%. When the CO_2% was in this range, the rate of production of methane for the entire period in the PAC (usually 60 mins) was greater than for initial period (usually) 30mins, as shown in Figure 3. Accordingly we reduced the duration of measurement to 20 and 40 minutes. Provided that CO_2% at the end of the measurement period does not exceed 4% (v/v) the estimates of rate of methane production measured over the consecutive time periods agree closely.

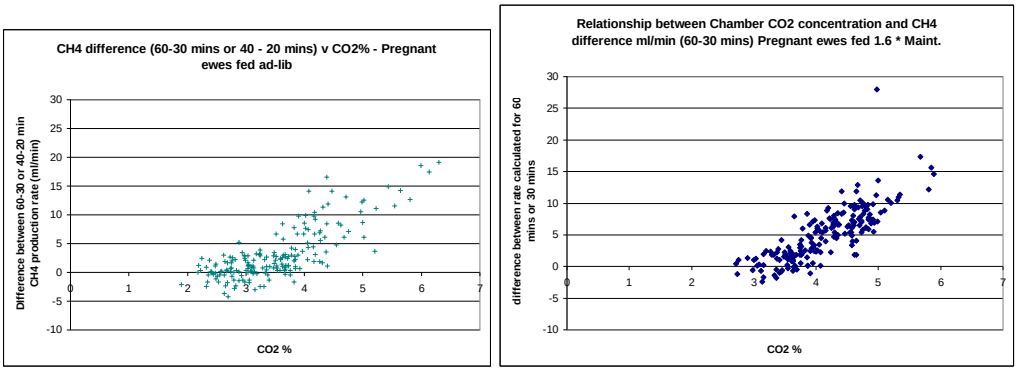


Figure 3. Relationship between difference in rate of methane production measured over entire period in PAC (40-60 mins) and half that period (20-30mins) and final CO2 concentration (% (v/v) in PACs. Animals were pregnant ewes (approx 115d pc), fed chaffed lucerne / oaten hay ad-lib (left pane) or restricted to 1.6* Maintenance requirements.

These observations indicate that measurement of gas exchange using PACs needs to be managed so that CO_2 concentration does not exceed 4% (v/v) at the end of the measurement period, For modest size sheep (40-50kg) eating a roughage based diet at maintenance a 60 min measurement period is suitable, but as the size of the animal (some were >75kg) and the feed intake (>2kg/d) increases, the risk that CO_2% will exceed 4% increases and the measurement period should be reduced. There are practical constraints to the sampling period set by time to load the sheep into the PACs, analysis time (measurement of methane using FID can be reliably achieved in less than 30 seconds, whereas measurement of CO_2 and O_2 concentrations using a FoxBox gas analyser take 75-90 seconds to stabilise). In our hands using 12 PACs this sets the minimum time between samples to 20 minutes.

4.3. WA growing and pregnant ewes

4.3.1. Ewe live weights

Live weights of ewes during the *ad libitum* feeding periods are shown in Figure 4. Average growth rates during *ad libitum* feeding were 214 g/day and 185 g/day for Group 1 and 2 respectively. The range in growth rates between sire groups that had more than 10 progeny was 202 to 242 g/day in Group 1 and 158 to 202 g/day in Group 2. At the times when methane was measured there was a 5.7 kg range in live weights between sire groups in Group 1 (36.5 *vs*. 42.2 kg) and a 6.4 kg range in live weights between sire groups in Group 2 (33.5 *vs*. 39.9 kg).

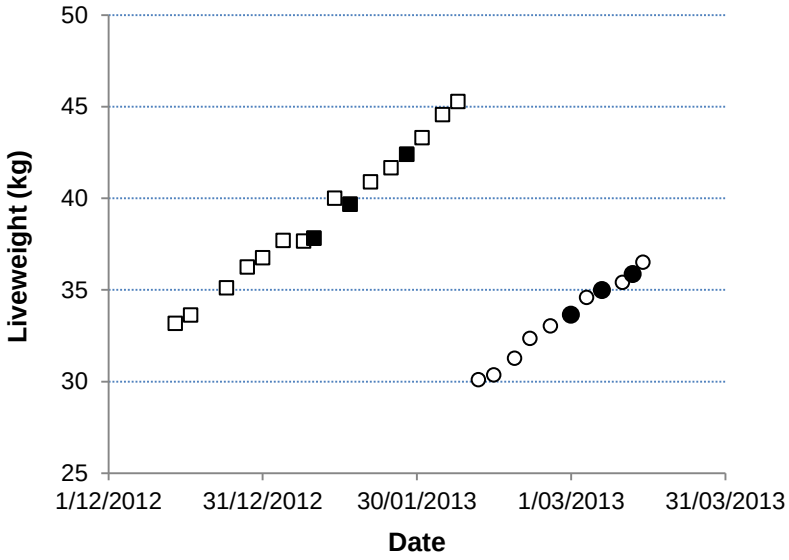
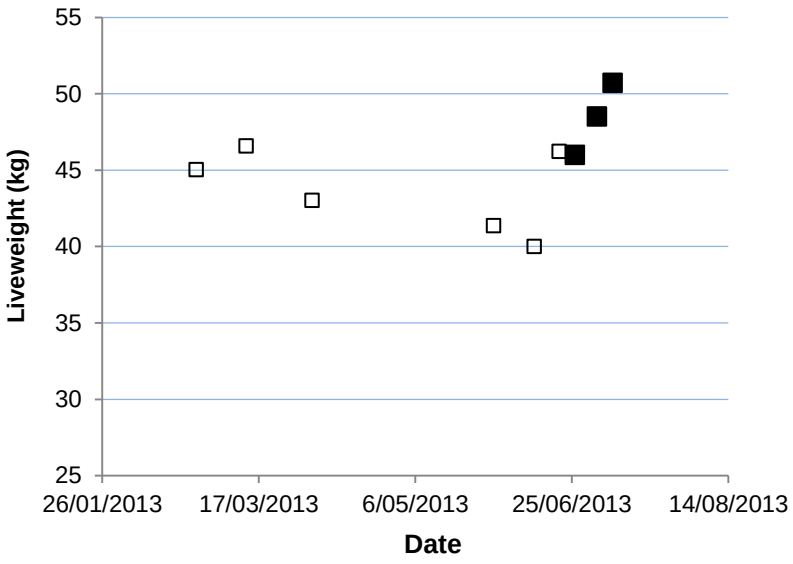
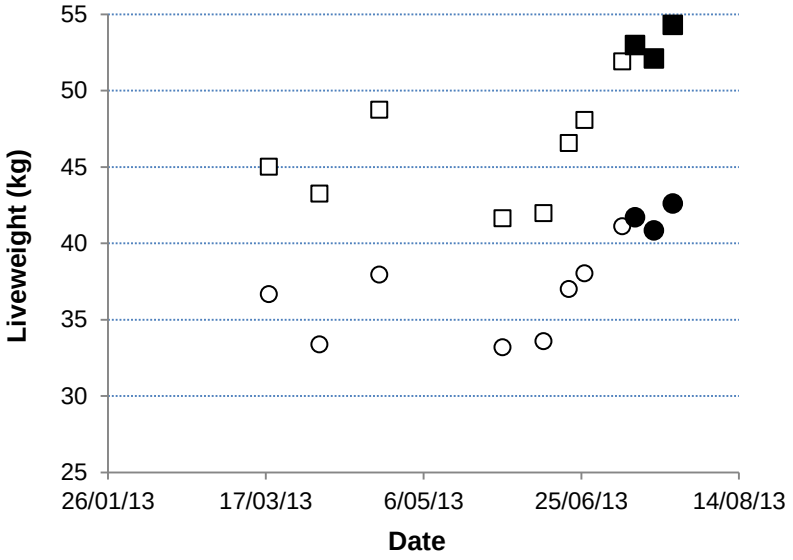


Figure 4. Live weights of Merino ewe weaners housed indoors and fed *ad libitum* high quality pellets over 55 days (Group 1) and 32 days (Group 2). Solid symbols represent the dates when methane production was measured using portable accumulation chambers.

The average live weight of groups A, B and C from joining until lambing are shown in Figure 5a, b and c respectively. The average live weight of ewes in all groups varied during pregnancy and as expected increased rapidly in late pregnancy due partly to weight of the conceptus. At the times when methane was measured during mid to late pregnancy there was a 8.5 kg range in live weights between sire groups in Pasture A (44.5 *vs* 53.0 kg), 5.3 kg range in live weights between sire groups (within ewe source) in Pasture B (43.3 *vs* 48.6 kg) and 3.7 kg range in live weights between sire groups in Pasture C (40.7 *vs* 44.4 kg).

Figure 5 (next page). Liveweights of Merino ewe weaners housed grazing similar pastures at Ridgefield; Pasture A (top); Pasture B (middle) and Pasture C (bottom). Ewe lambs from the Maternal Efficiency flock are represented by square symbols and ewe lambs from the Kojonup research flock are represented by circles. Solid symbols represent the dates when methane production was measured using portable accumulation chambers.


55
50
45
40
35
30
25
Liveweight (kg)
26/01/2013
17/03/2013
6/05/2013
25/06/2013
14/08/2013
Date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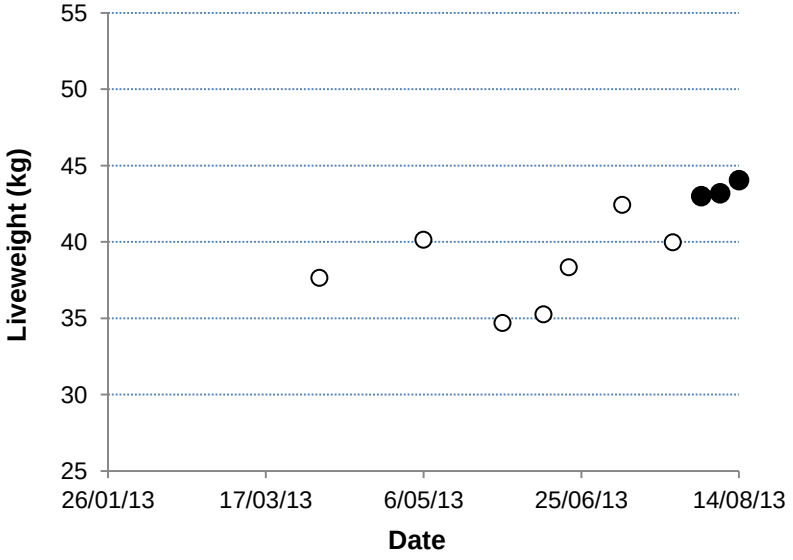
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4.3.2. Fertility and reproduction rates

A summary of the reproductive performance of the different groups of ewes is shown in Table 18. Across all ewes, 50% of the young ewes were dry and 50% were pregnant which was ideal for establishing the effects of pregnancy on the repeatability of methane measurements.

Table 18. Live weight at joining and the proportion of Merino ewe lambs scanned as dry or pregnant with a single or twin foetus for different groups.

Shed group	Pasture Group	Date at joining	Live weight at joining	Dry	Single	Multiple
1	A	27-Feb	45.2	35.1	53.6	11.3
	B	16-Mar	45.4	33.3	53.7	12.9
2	B	16-Mar	36.2	80.0	17.1	2.1
	C	4-Apr	37.8	57.1	39.3	**3.6**

Live weight at joining was related to reproductive rate (Fig. 6). There were no differences in average live weight or reproductive performance between GP1 ewe lambs from Pasture A or B, indicating that delaying joining by 17 days had no significant effect on reproduction. Delaying joining of GP2 ewes by 17-days increased reproductive performance however this is an artefact from preferentially sub-sampling a greater proportion of pregnant ewes in Pasture C to ensure an equal distribution of pregnant and dry ewes across all pasture groups.

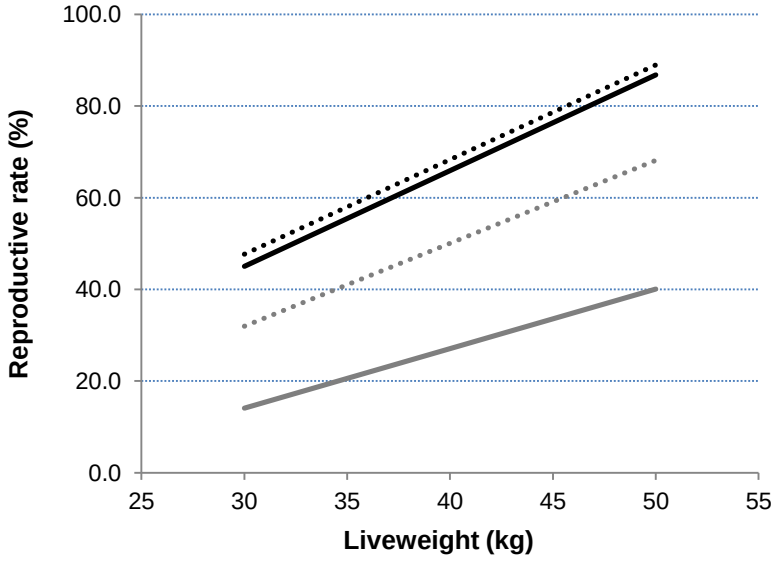

Figure 6. Relationship between live weight at joining and reproductive rate (foetuses/100 ewes joined) for group 1 (black) and group 2 (grey) ewes. The dashed line represents the response for ewe lambs joined 17-days after ewes represented by the solid line.

4.3.3. The effects of diet, reproductive performance and sire on methane production

Average methane production per day when ewe lambs were fed *ad libitum* indoors was estimated to be 44.6 L/day for Group 1 and 37.0 L/day for Group 2. This equated to 1.11 and 1.07 L methane/kg live weight per day, which is within the range of 1.03 to 1.34 L methane/kg live weight per day reported previously under similar feeding conditions (B.CCH.1015). Under grazing conditions, the average methane production varied from 21.9 to 26.9 L/day. This is likely to reflect changes in pasture conditions but that data has not been summarised.

There was no significant effect of pregnancy status or reproductive rate on methane production for ewes in Pasture A or C, but pregnant ewes produced more methane on Pasture B than dry ewes. The reason for this inconsistent response is not known. The methane production data from Pasture B required transforming, unlike that from Pasture A and C, so further analysis is required to confirm the variable responses to pregnancy status. The NSW study found no significant effects of pregnancy on methane production measured using portable accumulation chambers after adjustment for live weight and intake, regardless of feeding level. In the WA study the methane measurements were completed around day 120-125 of pregnancy so the absence of a consistent effect of pregnancy status probably means intakes were also similar.

On all occasions there was a significant (P<0.001) relationship between live weight and methane production. When live weight was included as a covariate in the analysis, pregnant ewes actually produced significantly less methane per day on all pastures compared to dry ewes (26.5 vs. 28.5 L/day, 21.3 vs. 23.0 L/day and 22.0 vs. 24.5 L/day for Pasture A, B and C), presumably because the live weight adjustment also included the weight of the conceptus rather than just maternal live weight.

Table 19. Effects of pregnancy status on methane production (L/day) for five groups of Merino ewes. Each ewe was measured three times. The values presented are not adjusted for liveweight.

		Status		*P value*		
Group	Mean	Dry	Pregnant	Pregnancy	Liveweight	state.sire
Indoors G1	44.6 ± 1.06	-	-	-	<0.001	-
G2	37.0 ± 0.68	-	-	-	<0.001	-
Pasture A	26.9 ± 2.39	26.2	27.6	0.195	<0.001	n.s.
B	21.9 ± 2.83	20.4	23.3	<0.001	<0.001	n.s.
C	23.6 ± 1.65	23.1	24.1	0.566	<0.001	n.s.

A summary of sire effects on methane production are shown in Table 20. For sires with more than 10 progeny, the range in the estimated daily methane production between sire groups was 12% under ad libitum feeding conditions (38.5 *vs*. 43.3 L/day) and 16% under grazing (22.0 *vs*. 25.7 L/day). When daily methane production was adjusted for live weight, the range between sire groups was only 9% under ad libitum feeding and 19% under grazing. There was no significant interaction between sire and pregnancy status or reproductive rate. This is consistent with the results from the NSW site and suggests that differences between sires are maintained across physiological states. However, unlike the results from the NSW site, there was a significant sire x diet interaction suggesting that different genetics could be

involved in methane production across different diets. This needs to be confirmed with a larger number of sires of markedly different intrinsic methane production rates.

Table 20. Summary of sire effects on number of progeny and REML predicted methane production (L/day).

Sire	Shed		Pasture	
	# progeny	Methane	# progeny	Methane
1	26	41.2	24	23.7
2	26	39.5	23	25.6
3	2	39.5	2	24.4
4	1	40.6	1	24.6
5	8	39.1	7	24.4
6	5	41.4	4	22.8
7	17	38.5	16	25.7
8	18	41.1	15	25.4
9	18	43.2	15	24.3
10	7	41.1	7	23.3
11	21	39.5	21	23.5
12	19	38.7	14	25.0
13	14	40.2	5	25.4
14	17	40.7	17	24.6
15	19	41.4	11	23.6
16	17	40.5	11	25.0
17	11	41.6	9	24.2
18	23	40.1	20	23.4
19	31	43.3	30	23.8
20	12	43.0	10	22.0
21	15	41.2	13	23.8
22	49	40.2	41	23.2

4.3.4. Repeatability of methane production

Methane production was measured three times on all 371 animals when fed ad libitum indoors and then again under grazing conditions. The average repeatability for 60 minute measurements was 0.42 to 0.46 for the two groups fed ad libitum indoors (Table 21). This is very similar to that reported in the Final Report for B.CCH.1015 and that determined in this study from the NSW site (see Table 17). On average the repeatability between measurements was higher for ewes grazing pastures, especially for those in groups A and C, but slightly lower when combined across different diets. The magnitude of the repeatability across diets is surprising given the sire by diet interaction. Furthermore, the decrease in repeatability when the data was combined across diets appeared to be larger in the WA study that the NSW, possibly reflecting larger changes the quantity and quality of the diet. The progeny in WA will be remeasured on dry feed in summer to further explore this sire x diet interaction and repeatability of measurements.

The repeatability tended to be similar for two versus three measurements per animals or for methane tests over 40 and 60 minutes (Table 21). The correlation between the two test periods averaged 0.97 (range 0.95 to 0.98; $P<0.001$), whereas the correlation was weaker for 60 min vs. 30 min or 20 min test periods (data not shown). A summary of Bartlett's test for homogeneity of the variances for different test periods is shown for the different groups of ewes in Table 22. All the 20 min test periods and some of the 30 min test periods had significantly greater variation than the 60 min test periods. There was no evidence against the assumption of equal variation between the 40 minute and 60 minute tests. We have previously shown that the heritability of methane production is similar for both 40 and 60 minute tests, but

the heritability estimate derived from a 20 minute test is about half that derived from the longer test periods (0.07 *vs*. 0.13; Thompson unpublished). Together with the data from NSW, where in studies using heavier animals eating more feed and at final CO_2 concentration in the portable chambers >4% there is an elevated rate of CH4 production, it appears that a test length of 40 minutes may be more suitable than 60 minutes. Of course, this depends on the weight and intake of the animals tested. Smaller sheep, eating less may well require 60 minute test period to maximise sensitivity of the assay.

Table 21. Repeatability of methane measurements for five groups of Merino ewes within and across different diets. The values are derived from three measurements per sheep of methane production in portable accumulation chambers over 20, 30, 40 and 60 minutes. Average repeatability for the 60 minute measurement based on two measures per sheep are also given.

Group	Number and duration of methane measurements				
	Three				Two
	20 mins	30 mins	40 mins	60 mins	60 mins
	without liveweight				
Indoors G1	0.30	0.34	0.38	0.42	0.42
G2	0.41	0.43	0.47	0.46	0.45
Pasture A	0.48	0.52	0.57	0.59	0.61
B	0.26	0.41	0.39	0.39	n.a.
C	0.23	0.49	0.51	0.59	0.57
Combined GP1 & A	n.a.	n.a.	n.a.	0.35	n.a.
Combined GP2 & C	n.a.	n.a.	n.a.	0.40	n.a.
	with liveweight				
1	0.16	0.20	0.23	0.26	0.27
2	0.21	0.25	0.27	0.24	0.22
a	0.37	0.38	0.42	0.43	0.45
b	0.20	0.30	0.28	0.24	n.a.
c	0.18	0.39	0.41	0.48	0.46
combined 1 & a	n.a.	n.a.	n.a.	0.19	n.a.
combined 2 & b	n.a.	n.a.	n.a.	0.19	n.a.

When the repeatability was adjusted for live weight, as a proxy for feed intake, the repeatability comboned across diets declines from about 0.40 to 0.2. This is similar to the NSW study and other work in NZ. The observation that repeatability of CH_4 production rate reduces as CH4 production rate is adjusted for other factors likely to be directly or indirectly related to methane production is expected. Interestingly, the repeatability even after adjustment for live weight was twice as high for ewe grazing Pastures A and C than when these ewes were fed indoors.

4.3.5. Feed intake and its relationship with methane production

Feed intake was significantly related to methane production for GP2 fed ad libitum pellets indoors (the analysis has not been completed for GP1 ewes). However, feed intake over the 24 hours prior to methane measurement explained only 20 to 30% of

the variance in methane emissions (P<0.001), with most variation in methane explained by intake over the 8 hours prior to methane measurements (Table 23). Feed intake during the period between 24 and 48 hours prior to methane measurements did not explain any variation in methane additional to that explained by intake during the 0 to 24 hour period. The variation in methane production explained by intake is considerably lower than was observed at the NSW site, probably reflecting real differences in diet composition and total intake but also greater errors in estimation of intake over short periods using the automated system in WA. Further work is still in progress to refine methods for cleaning the intake data collected from the automated feed intake facility in WA. Live weight explained about 35% of the variation in methane production and including live weight in the statistical model consistently explained an additional 20% of the variance in methane production over and above that explained by intake during the 24 hour period prior to methane measurements. At this stage there has been no attempt to derive methane yields. The data we have reported here will also be combined with records from an addition 1500 animals, including GP1, which should improve the accuracy of predictions.

Table 22. Homogeneity of variance (P value) between measuring methane production over 60 minutes compared to shorter period. The values are based on three measurements of each sheep under ad libitum feeding and three measurements under grazing conditions.

	20 mins	30 mins	40 mins
Indoors G1	0.001	0.03	0.41
G2	0.001	0.21	0.54
Pasture A	0.001	0.18	0.35
B	0.004	0.47	0.42
C	0.001	0.01	0.55

Table 23. Variation in methane production explained by live weight and feed intake over different periods prior to the measurement of methane. Sheep source was included in all models. Data is for 156 animals fed ad libitum pellets indoors and measured for methane production on three occasions.

Source	r-sq (blocking)	P-value
Liveweight (LW)	34.6	
Intake (0 - 4 hrs)	25.4	P<0.001
LW + intake (0 - 4 hrs)	45.5	P<0.001
Intake (0 - 8 hrs)	27.8	P<0.001
LW + intake (0 – 8 hrs)	44.7	P<0.001
Intake (0 – 12 hrs)	23.3	P<0.001
LW + intake (0 – 12 hrs)	41.7	P<0.001
Intake (0 – 24 hrs)	19.2	P<0.001
LW + intake (0 - 24 hrs)	38.2	P<0.001
Intake (24 – 48 hrs)	15.2	P<0.001
LW + intake (24 – 48 hrs)	36.4	n.s.

5. Discussion

The results in this report are by necessity preliminary. Work to establish a suitable protocol and to measure the pattern of methane production throughout the annual production cycle of ewes is ongoing, as was intended during the construction of this project. We have been successful in obtaining DAFF and ongoing MLA/AWI funds to complete this work, which we anticipate will be achieved by the second quarter of 2014.

5.1. *Establishing a suitable protocol for measurement of methane production*

The data collected so far suggest that using feed intake measures along with short term methane measures although useful is difficult to interpret and will be difficult to implement in practice. Methane yield (CH_4 production / feed eaten and fermented in the rumen) is variable depending on factors that affect the proportion of ingested feed fermented in the rumen. Protocols to control intake to a fixed proportion of live weight (or maintenance, or some other production function) are difficult to implement in the field. It is possible to implement such protocols in controlled environments, where methane yield measured over say 22-24 hrs can be achieved. Even under such controlled conditions the rate of methane production varies throughout the day primarily in response to variation in feed intake within the day. Evidence from other studies suggests that CH_4 production rate responds to feed intake within an hour, and may demonstrate Michaelis-Menton like saturation kinetics. It therefore seems to be a difficult ask to establish a standardised protocol for field measures of methane yield if feed intake is required to be measured. Not only is that almost impossible to achieve in field / grazing situations, the intake that is required to be measured is that within several hours of the methane measurement. Then intake must be controlled so that it provides a standard “dose” of fermentable nutrients. It is extremely unlikely that can be achieved.

Alternates might be available. We have used live weight and CO_2 production during the same time as methane measurements as covariates. CO_2 production rate and live weight accounted for approximately 63% of the variation in feed intake in the NSW study. CO_2 in particular can be measured at the same time as CH_4 and accounts for more variation than live weight (Table 17 above). We would anticipate that CO_2 is an index of metabolic rate including feed intake in the short term. CO_2 production rate could be used as a proxy for intake in analyses of CH_4 production rate. There are also other reasons for measuring CO_2, we found that when CO_2 concentration in the portable chambers approached 4% at the end of the measurement period, that the rate of production of CH_4 increased. We would recommend on the basis of the data to hand, that CO_2 be used to provide a quality control check on the CH_4 measurements.

We have not yet sufficient data to add to our estimates of genetic parameters. If we are unable to accurately estimate methane yield using the short term PAC methodology then two options are available. One is to abandon PACs and use respiration chambers only, with animals confined in an animal house prior to measurement and to tightly control feed intake before and during measurement of methane. The other is to use short term measures of CH_4 in the field with adjustment for some index of intake (CO_2 and / or live weight). The latter will be less precise, but has the advantage of markedly less cost and much higher throughput of animals.

The results we report here of estimates of repeatability for different ways of expressing a methane production rate are close to those reported elsewhere. Using

respiration chambers Pinares-Patino *et al.* (2013) have reported long term (across periods and years) repeatability of methane production (g/day) of 0.53-0.55 and of methane yield (g Ch_4 / kg DMI) of 0.24-0.26. Robinson and others (see for example final report B.CCH.1015) have estimated repeatability of CH_4 production rate adjusted for live weight measured using PACs on animals from field studies in the range 0.25-0.3. These are close to the estimates obtained here in both the NSW and WA studies across a range of pasture types, feed intakes and physiological states.

5.2. Preliminary results of methane production from pregnant sheep

The results obtained from the NSW sites show that there is a small effect of pregnancy status on methane production rate adjusted for feed intake when measured in respiration chambers. A reduction in methane production rate would be anticipated during pregnancy if there is a concomitant decrease in retention time of digesta in the rumen. Our results suggest there is a small reduction in methane production rate associated with number of fetuses after adjustment for intake and or live weight. The reduction in methane after adjustment for live weight, could also reflect that the live weights used included the weight of the conceptus. However, in both the NSW and WA studies there is no evidence of a sire * pregnancy status interaction on methane production rate. Furthermore, although there may be a reduction in methane production rate with pregnancy status, the magnitude of the effect was such that at present there is no case for adjusting the inventory to account for it. Additional measurements are planned to obtain comparable data during lactation and or again when the ewes are dry. This will provide additional data to investigate the need to adjust calculations used for inventory purposes for physiological state.

5.3. Indirect measures / indicators of methane production

There are theoretical reasons to believe that VFA production rate is in part a determinant of methane production, however there is no evidence in the current study that simply measuring VFA concentration can be used an indicator of methane production. This is similar to results reported elsewhere (McPhee and Hegarty, 2011). There is a suggestion that there may be genetic correlation between methane production rate and the proportion of VFA as propionate (Pinares-Patino *et al.* 2013, JC McEwan Pers Comm) in sheep and cattle (Herd *et al*. 2013). As yet we have insufficient evidence to confirm those observations. There was no sire effect on rumen volume after correction for live weight, and no significant difference between sire progeny groups in the proportion of different anatomical structures in the reticulo-rumen.

5.4. Implications of trait definition for developing selection methods

We have yet to explore the consequences of relaxing the target of methane yield in a breeding objective using Sheep Object. We believe we need to predict the genetic gain possible with less direct measures of methane production than methane yield. Modelling should provide us with a process to study the trade-offs between accuracy and numbers of animals required to achieve a selection outcome. However, the traits under selection pressure may well differ as a consequence of different measurement protocols. For example, selection on methane yield at a fixed intake of known feed (say maintenance) will most likely affect the rumen environment. Goopy *et al.* (2013) have shown that animals with high methane yield (estimated in respiration chambers at maintenance intake) have larger rumens and slower digesta retention times. Selection for lower methane yield could (if this mechanism was the predominant factor in reduce methane yield) have long term consequences on production in harsh

environments. We don't know what selection for CH_4 adjusted for say CO_2 production will affect, but it is likely to be different to selection on methane yield at fixed intake. The nature of phenotypic consequences of selection due to different traits are difficult to predict, but should be considered during trait development and looked for during early stages of selection studies..

6. Recommendations

This project was conducted as a step to developing improved and robust protocols for measurement of sheep to obtain genetic parameters for methane production. The first phase of the project is on track to complete as planned in mid-2014. By then we anticipate that a suitably robust protocol will have been established for implementation and testing in measurement of many more industry sheep from structured populations. The full project was successful in achieving support from DAFF Filling the Research Gaps Round 2, with additional support from MLA and AWI. Contracts and sub contracts are currently being established to enable continuation of this work to its planned conclusion.

7. Acknowledgements

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