REPEATABILITIES FOR METHANE EMISSION IN MERINO EWES ON PASTURE ACROSS DIFFERENT AGES

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SUMMARY

Methane production in sheep is a novel trait that requires the development of consistent measurement protocols. The objective of this study was to estimate repeatabilities for methane production adjusted for liveweight measured in portable accumulation chambers in Merino ewes on pasture. Repeatabilities were low to moderate. No improvements in accuracy of the phenotypic variance could be achieved by additional measurements. Most likely the trait expressed at different ages and in particular different physiological status was not the same in lactating and dry animals, but the analysis in this study was not able to support this hypothesis.

INTRODUCTION

Methane emission from livestock could in future pose a constraint on freedom to operate if green house gas emissions are capped. As a novel target trait for ruminant livestock systems it calls for the development of measurement methods that are beyond current industry practice. Ideally that includes not just the measure of methane production but also of predisposing factors such as feed quality and intake leading to the amount of fermentable substrate. The objective of this study is to produce background knowledge for the development of a measurement protocol for methane production of ewes on pasture. Repeatabilities of methane production were estimated at different ages and physiological states and the increase in accuracy of measurement through repeated records investigated.

MATERIALS AND METHODS

Data on methane production was collected on 96 Merino ewes at different ages. The times of measurements reflect not only a trajectory in age, but the animals also differed in their physiological state (Table 1). Sheep were measured twice at approx. 15 months of age with 3-4 weeks between the two measurements (Treatment 1 and 2 (T1 and T2)), twice as lactating adults at about 21 months of age with 2 weeks between measurements (Treatment 3 and 4 (T3 and T4)) and once as dry adults at around 27 months of age (Treatment 5 = T5). Measurements were repeated once for each treatment within 3 days except for T5.

Table 1. Treatment names, age and physiological status of experimental sheep and pasture availability and time of year (Date)

Treatment	Age	Reproductive status	Repetition	Date	Pasture availability (kgDM/ha)
T1	Yearling (15m)	Dry	2	February 2013	1500
T2	Yearling (15m)	Dry	2	March 2013	1800
T3	Adult (21m)	Lactating	2	Early November 2013	915
T4	Adult (21m)	Lactating	2	Mid November 2013	1100
T5	Adult (27m),	Dry	1	May 2014	1100

Animals were kept on pasture at the Glen Innes Research and Advisory Station in the New England area of New South Wales, Australia. Feed availability varied from 900 to 1800 kg total dry matter per hectare (DM/ha) (Table 1). Methane production was measured using portable accumulation chambers (PAC), which enable individual animal measures of methane production in the field over a short period of time (Goopy *et al*. 2011). For this study individual animals were confined to the PAC for 40 or 60 minutes (Table 2). Liveweight (LWT) was recorded immediately after gas measurement. Animals were removed from feed and water one hour before measurement. Twelve sheep were measured per run, four runs were conducted each day. Animals were randomly assigned to runs and chambers in the order they entered the race. Therefore, short-term repeat measures after 3 days within each treatment, were recorded in a different order. It was not possible to record feed intake.

Statistical analysis. A univariate animal model for repeated measures was fitted using ASReml (Gilmour *et al.* 2009) to estimate repeatability (r) of methane emission in sheep. The repeatability is the ratio between the permanent environmental or between-animal variance (V_{Eg}) and the phenotypic variance (V_P), which is the sum of V_{Eg} and the temporary or within-animal variance (V_{Es}) (Falconer and Mackay 1996). It was not possible to fit a meaningful additive genetic effect with only four sires and limited pedigree.

Repeatabilities were estimated within and across treatments. Fixed effect levels within treatment comprised: day of measurement, run, repetition, chamber number and number of lambs at foot (none, single or twin lambs) for T3 and T4. Liveweight was fitted as a covariate to adjust for potential variation in feed intake and rumen volume. An identity matrix for the animal effect was fitted as random.

For the sake of comparison, we hypothesised that CH_4 in adult ewes (T5) is the most suitable measure to relate to lifetime CH_4 production, which was ultimately the trait that will become the breeding objective. The improvement in accuracy of phenotypic measurement is evaluated by adding measurements as lactating (T3 and T4) and young sheep (T1 and T2). Improvement was assessed by the associated effect on the phenotypic and environmental variances.

RESULTS AND DISCUSSION

CH_4 production is due to fermentation of feed in the rumen (Blaxter and Clapperton, 1965). As a consequence CH_4 production is expected to increase when more feed is ingested due to increased feed on offer or by increasing energy demand, e.g. lactation. Data was adjusted for liveweight, which was significant as covariate, but mean total CH_4 production differed significantly between treatments (Table 2). Total CH_4 production was highest during T3 and T4, despite low feed availability, because of increased feed intake due to the animals lactating during that time. Higher CH_4 production also occurred during T2 compared to T1 because more feed was on offer. Mean CH_4 production was the lowest for T5, most likely due to lowest intake as a consequence of amount of feed on offer and the ewes neither growing nor being pregnant or lactating.

Table 2. Descriptive statistics for methane production in Merino ewes at increasing age at pasture (mmol CH_4/min). Time = time period (mins) over which CH_4 was measured

Treatment	Time	No of records	Mean	Min	Max	StdDev
T1	60	192	0.91	0.38	1.59	0.23
T2	60	192	1.12	0.54	2.11	0.25
T3	40	192	1.32	0.55	2.47	0.38
T4	40	192	1.61	0.47	2.85	0.41
T5	40	96	0.84	0.39	1.30	0.22

Heritabilities (h^2) for methane production in sheep have been reported at h^2 ~0.29 for gCH4/day, 0.13 for gCH4/kg feed measured in respiration chambers (Pinares-Patino *et al.* 2013) and ~0.1 for gCH4/day adjusted for LWT measured in PACs (Robinson *et al*. 2014). Given the low to moderate heritabilities for methane related traits, the repeatability, which is the upper limit for the heritability, was expected to be moderate.

Repeatability with treatments. This study established that short term repeatabilities, measured within 3 days, for CH_4 adjusted for LWT were moderate for ewes at yearling age (r(T1)=0.33$\pm$0.09 and r(T2)=0.37$\pm$0.09). During lactation the repeatabilities were moderate (r(T4)=0.40$\pm$0.09) to high r(T3)=0.62$\pm$0.06. The increase in repeatability was due to higher between-animal and consequently phenotypic variance. In T5 all animals were only measured once and short term repeatabilities could not be established. The repeatability estimates were lower than repeatabilities from respiration chamber data on consecutive days (r = 0.94 $\pm$ 0.003, Pinares-Patino *et al.* 2013), which demonstrates the influence of controlled feed intake and highlights the problematic adjustment for LWT, as was done in this study.

Table 3. Repeatabilities and variances for CH_4 emission adjusted for LWT at different ages

Treatment	Repeatability	V_P	V_{Eg}	V_{Es}
T1 & T2	0.25 $\pm$ 0.07	0.027	0.007	0.020
T1 & T3	0.26 $\pm$ 0.06	0.039	0.010	0.029
T1 & T4	0.28 $\pm$ 0.05	0.047	0.013	0.034
T1 & T5	0.17 $\pm$ 0.05	0.029	0.005	0.024
T2 & T3	0.32 $\pm$ 0.08	0.034	0.011	0.023
T2 & T4	0.20 $\pm$ 0.05	0.043	0.009	0.034
T2 & T5	0.27 $\pm$ 0.06	0.026	0.007	0.019
T3 & T4	0.40 $\pm$ 0.07	0.057	0.023	0.034
T3 & T5	0.38 $\pm$ 0.06	0.037	0.014	0.023
T4 & T5	0.30 $\pm$ 0.06	0.047	0.014	0.033

Repeatability across/between treatments. Repeatabilities for CH_4 production adjusted for LWT across treatments, measured at least one month apart were low to moderate (Table 3). The estimates were lower than estimates reported by Pinares-Patino *et al*. (2013) of r=0.55$\pm$0.02 for gCH4/day, but align with estimates of r=0.25 for gCH4/day adjusted for LWT measured in PACs reported by Robinson *et al.* (2014). Repeatabilities are slightly higher at later ages, which was due to an increase in between-animal variance.

Low repeatabilities indicated that the accuracy of CH_4 measurement with PACs on animals from pasture would benefit from repeated measures. As outlined earlier, CH_4 emission at T5 was assumed to be the representative trait of life time CH_4 emission. It was investigated if the measures at different treatments were appropriate to add as repeated measures to increase the accuracy of the phenotypic variance. The results in Figure 1 demonstrate that any of the other treatments are unsuitable as repeated measures to increase the accuracy of phenotypic variance for CH_4 production in T5. CH_4 production in lactating ewes (T3 and T4) added variance, mainly through an increase in the within-animal variance. This could indicate that CH_4 production adjusted for LWT is a different trait in dry and lactating ewes. It also demonstrates that LWT might not be an appropriate adjustment for feed intake. This makes sense because lactating ewes would eat more and produce more CH_4 compared to dry ewes at the same LWT. A small decrease in phenotypic variance was observed by combining T5 and T2, but the addition of either T2 or T1 decreased the between-animal variance, which again, might be a reflection of a smaller additive genetic variance for T1 and T2 than T5. Differences in magnitude of the CH4 measurements between the treatments would have contributed to the lower repeatability estimates when treatment data is added.

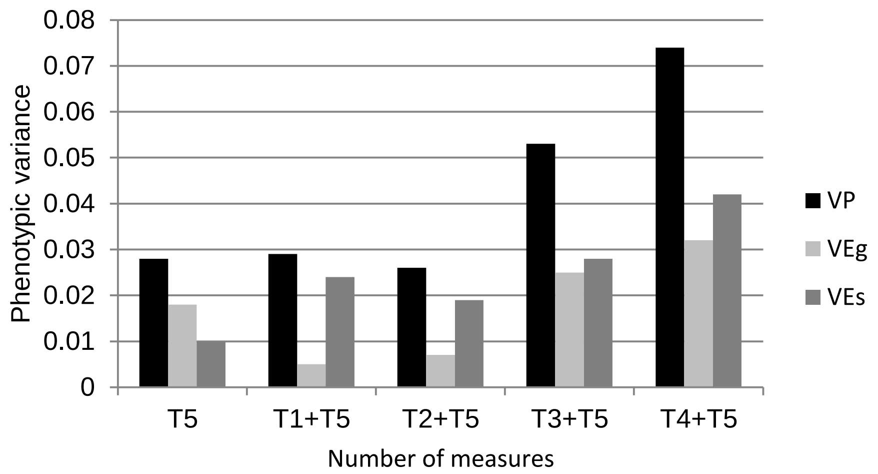

Figure 1. Phenotypic (V_p), permanent environmental (V_{Eg}) and special environmental variance (V_{Es}) with increasing number of measurements.

CONCLUSION

It is suggested that a measurement protocol for CH_4 production in Merino ewes on pasture in young or pregnant sheep is not a reliable indicator of adult performance. However, this data relates only to CH_4 adjusted for LWT and ignores the poor relationship between feed intake and LWT. A more desirable and appropriate phenotype for CH_4 production would account for the amount and quality of feed eaten, such as methane yield. However, it is not possible to measure feed intake with PACs in the field.

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