BRIEF COMMUNICATION: Effects of measuring methane in pregnant ewes using portable accumulation chambers on pregnancy outcomes and lamb performance to weaning

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Abstract

The portable accumulation chamber (PAC) method provides an on-farm methane measurement over a short timeframe that is scalable to measure large numbers of animals. The aim of this study was to assess the safety of use of PAC in pregnant ewes. Methane production was measured in ewes (n=40 per group) using PAC at approximately 70 or 100 days of gestation. Control animals were either exposed to yarding and sorting only (Days 70 and 100) or exposed to yarding, sorting and restriction from feed as outlined in the standard operating procedure for PAC (Day 100 only). Ewe health was not differentially affected by PAC measurement. Confinement in PAC did not affect (P>0.50) lamb survival, with the proportion of lambs weaned relative to number at mid-pregnancy ranging between 0.817 to 0.898. Birth and weaning weights of the lamb were similar (P>0.28) between all groups (birth weight ranged between 4.8 to 5.1 kg, weaning weight ranged between 28.1 to 29.3 kg). Carbon dioxide levels in PAC were not elevated compared to what has previously been observed in non-pregnant ewes. Methane concentrations were calculated for the ewes around day 100 of pregnancy and the average methane production was 23.2 ± 5.8 g per day. In conclusion, PAC appears to be a safe method for measuring methane traits in ewes during the middle to later stages of pregnancy.

Keywords: PAC methane measurements; Pregnant ewes; Lambing performance

Introduction

Methane production by ruminant animals is recognised as a significant contributor to greenhouse gas emissions and global warming. Globally, enteric methane production from ruminant's accounts for around 6% of anthropogenic greenhouse gas emissions (Beauchemin et al. 2020). The contribution that enteric methane makes to New Zealand's greenhouse gas emissions is even higher, accounting for over 35 % of total emissions (Ministry for the Environment 2023). Reducing enteric methane emissions from ruminants is seen as essential to allow NZ, and the world, to meet emissions reduction targets.

A key aspect of developing approaches to reduce enteric methane production is being able to accurately measure methane production. This allows assessment of the effects of diet, feed additives, genetics, and other products on methane production to enable development of the most effective approaches for reducing methane. Multiple methods have been developed to measure methane production, with the portable accumulation chamber (PAC) being recognised for its ability to measure a large number of sheep in a short timeframe (Goopy et al. 2011; Jonker et al. 2018). These chambers can be scaled to include multiple units on a single trailer, making the measurement of 80+ sheep in a single day with a single trailer containing 12 units feasible. A single measurement of less than an hour in duration can be used to provide measurements of methane production suitable for use in genetic selection programmes, which are currently being commercially applied in New Zealand (Lawrence 2022).

Breeding females account for a significant proportion of farm greenhouse gas emissions. Additionally, ewes are pregnant for ~40% of the year. Thus, it is important to understand if pregnancy affects methane production and potential interactions between pregnancy and methane mitigation approaches. To do this, methods to measure methane production in pregnant ewes, without compromising the health of the dam or offspring, need to be identified.

Use of a PAC requires the animal to be held in a sealed environment for the duration of the time that the measurement is made (Goopy et al. 2011; Jonker et al. 2018). During this timeframe, oxygen is limited to that originally in the chamber at the time of sealing. Currently, PAC's have been primarily utilised on young growing lambs (prepubertal), with some adult male and non-pregnant females also being measured without incident. However, it is known that longer term oxygen deprivation, particularly later in gestation, can have negative impacts on the pregnant female, and particularly her offspring (Morrison 2008).

Our aim was to determine if a single PAC measurement would affect the ewe's or her offsprings' health. A secondary aim was to measure methane production in pregnant ewes and determine if number of foetuses affected methane production.

Materials and methods

The experiment was run in accordance with the New Zealand Animal Welfare regulations and approved by the AgResearch Animal Ethics Committee (application number 1912).

Ewes were selected from a group of mature (2.5-4.5 years of age) breeding ewes with recorded mating marks (recorded every Monday, Wednesday, Friday). A premating weight was obtained on the first day of the breeding period. Transabdominal ultrasound scanning was utilised to determine pregnancy status including litter size. All animals were managed as a single group on pasture and yarded at each PAC measurement for sorting of animals. Four groups of ewes (n=40 per group) were selected : ewes yarded but returned to pasture (65-77 days of gestation for first PAC measurement and 95-107 for second PAC measurement; Control), underwent a PAC measurement between 65-77 days of gestation (PAC mid-pregnancy), underwent a PAC measurement from 95-107 days of gestation (PAC later pregnancy), or were yarded, retained in the yards (thus restricted from access to feed) but not exposed to the PAC at 95-107 days of gestation (Feed restriction). All ewes underwent another transabdominal ultrasound scan approximately one month after the initial pregnancy scan. Following assigned treatment, all ewes were fed on pasture or crops to meet their requirements as assessed by experience farm staff. A fully recorded lambing was undertaken on these animals which included assigning lamb to dam and obtaining a birth weight and determining sex of the lamb within 24 hours of birth. Status of the lamb (alive or dead) was recorded within 24 hours of birth and at weaning. Weaning weight was also recorded.

Portable accumulation chambers were used to measure methane as previously described (Jonker et al., 2018). At each measurement timepoint, all ewes were measured for methane production on the same day. For each measurement day, a total of 6 rounds of measurements, with 6-7 animals per measurement round, were undertaken. Animal number per round were reduced from the typical 12 to allow more frequent monitoring of the pregnant animals given the uncertainty of their reaction to the chambers. Time off feed ranged from approximately 2 to 4 hours.

A total of 6 animals were removed from the analysis due to animal health problems associated with normal lambing (1 ewe from each group died before lambing) or lambing date was not recorded (1 ewe in the PAC mid-pregnancy group and 1 ewe in Feed restriction group). Foetal age for all groups was calculated using the observed mating mark. The following ewe traits were calculated. The proportion of lambs born relative to the number of lambs at scanning (PLB). The proportion of lambs born alive relative to the number of lambs at scanning (PLBA). The proportion of lambs weaned relative to the number of lambs at scanning (PLW). The proportion of lambs weaned relative to the number of lambs born alive (PLLW). The power to detect a 20% drop in survival for a treatment relative to the control was between 61.2% (PLW) and 82.9% (PLB).

Lambs were only fostered to other ewes if an experienced shepherd determined that leaving the lamb with the mother was likely to result in the lamb's death (dam abandoned lamb, dam was not providing sufficient milk for lamb). For the purpose of the maternal traits, lambs that were removed from the dam were considered to have died between birth and weaning. The following lamb traits were assessed. Survival of a lamb born alive to weaning (LSW), the weight of the lamb around birth whether the lamb was alive or dead (BWt) and the weight of the lamb at weaning (WWt). For lamb traits, a lamb that was fostered onto another ewe was retained in the data set. Difference in lambing date were calculated by determining the deviation (in days) in the birthdate relative to the mean birthdate of all animals (bdev).

All statistical analyses were carried out in R. Differences in age, number of foetuses present at mid-pregnancy scanning, liveweight of the ewe just prior to joining with the ram, foetal age, and the birth date deviation from average birth data between groups was analysed using ANOVA (Table 1). A generalised linear model (binomial) was fitted for the ewe traits, with a generalised linear mixed model (binomial) used for the lamb trait LSW and linear mixed model (gaussian) used for the BWt and WWt lamb traits. For the maternal traits PLBA, PLW and PLLW, the glm function in base R was used, with the brglm2 model used for PLB given one group did not lose any lambs between scanning and birth. In addition to group, other fixed effects included in the model for all maternal traits were the age of the ewe, the weight of the ewe prior to joining with the ram, and the average birth weight of her lambs. In addition to group, other fixed effects included in the model for LSW were age of dam, number of lambs born and sex of the lamb with dam being fit as a random effect. For BWt, other fixed effects included bdev, number of lambs born, weight of ewe just prior to joining, sex of the lamb with dam being fit as a random effect. The model for WWt included all factors fitted for BWt as well as the average birth weight of all lambs from its dam.

PAC data was processed as described (Jonker et al., 2018) and analysed using linear models. For analysis of methane production, the fixed effects in the model included age of the ewe, time off pasture, CO2 concentrations, number of lambs scanned and foetal age. For analysis of carbon dioxide measurement, the fixed effects in the model included age of the ewe, time off pasture, liveweight at time of PAC measurement, number of lambs scanned and foetal age.

Results

There was no evidence of an effect of PAC measurements on any of the ewe traits analysed ($P>0.50$; Table 2). Considering all lamb mortality from scanning to weaning (PLW), lamb survival was on average greater than 80% for all groups (Table 2). Little foetal loss was noted between pregnancy scanning and time of birth (PLB) with no factors included in the model being shown to affect this trait ($P>0.95$). Between scanning and birth, including losses during birth (PLBA), showed that mortality, was less than 8% for all groups. Loss between birth and weaning, considering only lambs that were born alive (PLLW), was less than 12% for all groups and no factors included in the model affected this trait ($P>0.05$). There was no evidence of a negative effect of PAC measurement on any of the lamb traits analysed (Table 2).

Table 1: Mean (±sem) age, number of foetuses at mid-pregnancy (NFMP), the weight of the ewe at joining of the ewes and rams (Ewe Wt), foetal age at time of first PAC measurement (foetal age) and the deviation (in days) in the birthdate relative to the mean birthdate of all lambs (bdev) are presented.

	Group				
Variable	Control	PAC mid-pregnancy	PAC later pregnancy	Feed restriction	P-value
Age	3.82 ± 0.91	3.82 ± 0.93	3.77 ± 0.87	3.76 ± 0.88	0.988
NFMP	2.03 ± 0.54	1.95 ± 0.57	2.03 ± 0.63	1.97 ± 0.54	0.909
Ewe Wt	65 ± 6.74	65.4 ± 6.33	63.8 ± 6.59	64.4 ± 5.25	0.705
Foetal age	69.8 ± 3.75	69.4 ± 3.82	69.6 ± 3.89	69.4 ± 4.02	0.953
bdev	-0.17 ± 4.12	0.12 ± 4.58	-0.01 ± 4.33	0.07 ± 4.61	0.993

Survival of lambs that were alive at birth to weaning was greater than 90% for all groups and no factor included in the model was shown to affect this trait (P>0.05). Average birth weight of the lambs ranged from 4.8 to 5.1 kg. While PAC measurement did not affect birth weight, all other factors included in the model had a significant effect (P<0.05). Average weaning weight ranged from 28.1 to 29.3 kg. While PAC measurement did not have a significant effect on weaning weight, and ewe weight at joining only approached significance (P<0.10), all other factors included in the model significantly affected weaning weight (P<0.01).

Methane concentrations were calculated for the ewes around day 100 of pregnancy and the average methane production was 23.2 ± 5.8 whereas carbon dioxide averaged 1390.7 ± 281.8 g per day with an average time off feed of 2.64 hours. With the limited sample size available, no differences (P>0.15) among ewes with different litter sizes and no effects of gestational age were observed for either methane or carbon dioxide measurements.

Discussion

The current study provides evidence that methane measurement using PAC around mid-gestation or approximately 6 weeks prior to birth was not detrimental to either the ewe or the lamb. Given the restricted air exchange during the PAC measurement, there was concern that reduced oxygen availability might affect the pregnancy/lambs. Severe hypoxia late in gestation (day 131) through occlusion of the umbilical cord for just 10 minutes has been reported to result in preterm labour and delivery in ewes (Baburamani et al. 2021). Foetal blood oxygen levels are correlated with weight of the foetus during near term (day 139-142 of gestation) (Flouri et al. 2022). Previous work has shown that long term hypoxia during later pregnancy, such as that caused by undernutrition and/or carrying of multiple foetuses, was associated with reduced foetal weights (Sales et al. 2018).

However, there was no evidence that any ewe aborted their pregnancy following PAC measurements and partial loss of multiple pregnancies was very low and not different among groups. Similarly, birth date deviation did not differ between groups indicating that gestational length was similar in the groups of ewes. The birth weight of the lambs was not reduced by exposure to the PAC and their growth patterns were normal. Lamb loss was similar among groups throughout the period of mid-gestation through to weaning. Taken together, this provides evidence that the lambs born following measurement of methane in the PAC were not affected by the potential limitation of oxygen supply. Concentrations of methane and carbon dioxide produced per day was roughly similar, albeit that methane concentrations appear slightly greater to what is observed in non-pregnant ewes, (Jonker et al. 2018; Johnson et al. 2022) with no

Table 2. The table of means and 95 % CIs for each trait for each group. (See text for definition of abbreviations).

	Group [Means (CI)]				
Trait	Control	PAC mid-pregnancy	PAC later pregnancy	Feed restriction	P-value (Type III)
PLW	0.898 (0.805, 0.949)	0.865 (0.762, 0.928)	0.857 (0.753, 0.921)	0.817 (0.704, 0.893)	0.553
PLB	0.994 (0.927, 1.000)	0.979 (0.904, 0.996)	0.986 (0.922, 0.998)	0.966 (0.887, 0.991)	0.963
PLBA	0.932 (0.848, 0.971)	0.928 (0.841, 0.969)	0.946 (0.868, 0.979)	0.925 (0.836, 0.968)	0.945
PLLW	0.951 (0.866, 0.983)	0.914 (0.812, 0.963)	0.904 (0.804, 0.955)	0.884 (0.778, 0.944)	0.526
LSW	0.942 (0.706, 0.991)	0.941 (0.712, 0.991)	0.908 (0.667, 0.980)	0.917 (0.656, 0.985)	0.874
BWt	5.0 (4.69, 5.36)	4.8 (4.48, 5.16)	5.1 (4.77, 5.39)	5.0 (4.62 ,5.28)	0.285
WWt	28.1 (26.18, 29.92)	29.0 (27.10, 30.93)	29.1 (27.44, 30.83)	29.3 (27.40, 31.15)	0.378

observable effect of number of foetuses the ewe was carrying or foetal age. However, it should be noted that the number of animals in the current study was relatively low with a limited gestational range and further research to better understand the effects of differing litter size and gestational age would be warranted.

Measurement of methane using the portable accumulation chambers during mid-pregnancy up to approximately day 110 of gestation does not appear to negatively affect pregnancy outcome for the ewe or lamb survival or growth. PAC provides a useful tool to better understand methane production of the ewe throughout the year including during pregnancy. This can provide a tool to obtain a better understanding of the interaction of pregnancy with methane production as well as any methane reduction strategies. It should however be noted that this trial contained a relatively small number of animals and continued monitoring of the effects of use of PAC in larger numbers of pregnant females would be prudent.

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