BREEDING FOR LOW METHANE IS ASSOCIATED WITH LONGER DAYS TO CALVING IN FIRST-PARITY ANGUS FEMALES

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SUMMARY
Angus heifers (n=497) that had been divergently selected for methane yield (MY) were used to examine the impact of selection for MY on reproductive performance. Joining records for first-parity heifers over 3 different birth years were used to assess pregnancy rate, days to calving, calving rate, weaning rate, weight of the subsequent calf at birth and weaning, and weight of the female at calf weaning. Selection for MY did not have any impact on pregnancy rate, or weight of the subsequent calf at birth or weaning. However, Low MY heifers calved later in the season and were lighter in weight at calf weaning than High MY heifers. Evidence from this study, along with other studies, indicates that selection for Low MY is associated with calving later in the calving season due to a delay in the onset of puberty in Low MY heifers. Further investigations are needed to confirm any association between calving and weaning rates and genetic variation in MY and to gain better understanding of impact of selection for methane yield on lifetime reproductive performance.

INTRODUCTION
The contribution of livestock to worldwide greenhouse gas emissions is significant (14.5%) and primarily from ruminants (Gerber *et al.* 2013). Methane emission traits have been reported as moderately heritable in sheep (Pinares-Patino *et al.* 2011) and beef cattle (Donoghue *et al.* 2016a). While selection for low methane genotypes is now possible, the impact of this selection on female fertility traits is unknown. The objective of this study was to investigate whether differences exist between heifers from divergent methane yield selection lines for reproductive performance traits recorded during their first parity. These traits include pregnancy, calving and weaning rates, days to calving, weight of calf at birth and weaning, and weight of heifer at weaning of first calf.

MATERIALS AND METHODS
The females used in this project were heifers from the methane yield selection lines within two research herds of Angus cattle at the Agricultural Research Centre, Trangie, NSW, Australia. Methane yield (MY) is the amount of methane emitted per day divided by the weight of feed eaten on a dry-matter basis (g CH_4/kg DMI). Details on the creation of the selection lines and measurement of methane emissions can be obtained from Donoghue *et al.* (2016a). Females were born in 2009 (n=197); 2011 (n=177) and 2012 (n=123). All females available each year were joined at an average age of 500 days, except for heifers born in 2009, who were joined at approximately 780 days of age. Heifers were exposed to bulls for 9 weeks, in single sire mating groups at a ratio of 30 females per bull. The females were pregnancy tested by trans-rectal ultrasonography approximately 10 weeks after the end of joining. Only data from the females' first joining season was considered in this study.

Reproductive performance of the heifers was assessed by pregnancy rate (PREG), calving rate (CALV) and weaning rate (WEAN), defined as the percentage of heifers that were pregnant, percentage of heifers that calved, and percentage of heifers that weaned a calf, respectively, out of

the total number exposed to a bull. These rates were coded as binomial traits (e.g. 0, non-pregnant; 1, pregnant). Age and weight of the females at joining were also recorded.

Days to calving (DTC) was also studied as one of the reproductive performance traits, and was calculated as the number of days between the start of the joining season and the date of birth of the subsequent calf. Females that failed to calve were assigned a penalty value which was 21 days greater than the last female to calve in their contemporary group. Weight of the calf at birth and weaning, and weight of the female at the weaning of her calf were also recorded.

Data for weight traits were collected throughout the life of the females, including birth (BWT), weaning (WWT), yearling (YWT) and final (FWT) weight, which were measured at birth and at mean (±SD) age of 231 (±25), 422 (±23), and 603 (±79) d, respectively. In addition body composition traits were measured at mean (±SD) age of 585 (±97) d, and comprised ultrasound P8 fat depth (P8), eye muscle area (EMA) and intramuscular fat % (IMF). Within herd MY Estimated Breeding Values (MY EBV) were obtained for all heifers.

Model of analysis. All the traits, except the binomial traits (PREG, CALV and WEAN) were analysed using the Mixed Model procedure (PROC MIXED) of SAS (SAS Institute, Cary, NC). The model used for the analysis of weight (BWT, WWT, YWT and FWT), body composition (P8, EMA and IMF) traits, joining age (JOINAGE), joining weight (JOINWT) and weight of female at weaning of calf (MCWT) included the fixed effects of selection line (Low or High line), herd (Trangie1 or Trangie2) and birth year (2009, 2011 or 2012), the random effect of sire of heifer and the random residual error. Age of the heifer was included as a covariate in the model for all traits except BWT and JOINAGE, while age of dam was included in the model for BWT and WWT. Interactions between fixed effects were included when significant ($P < 0.05$).

For DTC, the model included the fixed effects of selection line and contemporary group, the random effect of sire of heifer and random residual error. Contemporary group for DTC included all females joined to the same sire in the same year. For calf birth (CALFBWT) and weaning (CALFWWT) weight, the model included the fixed effects of selection line and birth year, sex of the calf, the random effects of sire of the calf, sire of the heifer and the random residual error. Age of the heifer was included in the model for both traits, while age of the calf was included in the model for CALFWWT. Interactions between fixed effects were included when significant ($P < 0.05$).

The binomial traits (PREG, CALV and WEAN) were analysed using the Generalised Linear Model (PROC GENMOD) procedure of SAS with a logit link function. The model fitted included the fixed effects of selection line and contemporary group, sire of heifer and the random residual error. Contemporary group was as defined for DTC. Age at calving was also included in the model for CALV. Correlations between reproductive performance traits and weight, body composition and methane traits were examined using the CORR procedure in SAS.

RESULTS AND DISCUSSION

Least-squares means for weight, body composition traits and MY EBVs are presented in Table 1. There was a significant divergence between selection lines in MY EBV, being 0.20g CH_4/kg DMI ($P < 0.0001$; Table 1). There were no significant differences between MY selection line heifers for weight traits or EMA (all $P > 0.05$; Table 1). Bird-Gardiner *et al.* (2016) analysed a larger data set from the same project that included both males and females, and also reported no significant differences between MY selection lines for weight traits. Heifers from the Low MY selection line were significantly fatter at the P8 site ($P < 0.05$) and had significantly higher IMF% ($P < 0.05$) than heifers from the High MY selection line, which was unexpected given that the genetic corrections with MY reported by Donoghue *et al.* (2016a) were not statistically significant.

Table 1 Least-squares means (±SE) of weight and body composition traits and methane yield EBVs of heifers from divergent selection lines for methane yield

Trait	Selection Line		Significance[a]
	Low Methane Yield	High Methane Yield	
Number of heifers	227	270	-
Birth Weight (kg)	33.0 (0.3)	32.9 (0.2)	ns
Weaning Weight (kg)	229.8 (5.3)	232.8 (5.2)	ns
Yearling Weight (kg)	343.3 (2.0)	344.9 (1.8)	ns
Final Weight (kg)	419.3 (2.2)	421.8 (2.1)	ns
P8 fat (mm)	9.9 (0.6)	9.0 (0.6)	*
EMA (cm^2)	59.8 (0.5)	59.5 (0.5)	ns
IMF (%)	5.3 (0.09)	5.1 (0.09)	*
MY EBV[b] (gCH_4/kg DMI)	-0.12 (0.02)	0.08 (0.02)	**

[a] ** Significance at $P \leq 0.0001$; * Significance at $P \leq 0.05$; ns Non-significance at $P > 0.05$
[b] Methane Yield Estimated Breeding Value

Least-squares means for reproductive performance traits are presented in Table 2. There were no significant selection line differences in any of the reproductive performance traits of the heifers ($P > 0.05$; Table 2), except for DTC and MCWT where heifers from the Low MY selection line calved significantly later and were significantly lighter in weight at weaning of their first calf than heifers from the High MY selection line ($P < 0.05$; Table 2).

Table 2 Least-squares means (±SE) for transformed (T) and untransformed (UT) values of reproductive performance traits of heifers from divergent selection lines for methane yield

Trait	Data type	Selection Line		Significance[a]
		Low MY	High MY	
Number of heifers joined	-	227	270	-
Age at joining (days)	-	604.5 (1.3)	601.4 (1.2)	ns
Weight at joining (kg)	-	419.2 (2.6)	420.8 (2.6)	ns
Pregnancy Rate (%)	T	1.95±0.28	2.41±0.31	ns
	UT	86.6	91.1	-
Calving Rate (%)	T	1.80±0.43	2.21±0.45	ns
	UT	80.6	85.6	-
Days to Calving (days)	-	301.4 (1.2)	298.3 (1.1)	*
Calf Birth Weight (kg)	-	31.8 (0.3)	31.5 (0.3)	ns
Weaning Rate (%)	T	1.16±0.21	1.30±0.21	ns
	UT	75.8	78.2	-
Calf Weaning Weight (kg)	-	226.0 (9.5)	229.7 (9.6)	ns
Cow Weight at Weaning (kg)	-	499.3 (5.6)	510.6 (5.3)	*

[a] ** Significance at $P \leq 0.0001$; * Significance at $P \leq 0.05$; ns Non-significance at $P > 0.05$

Phenotypic correlations between selected weight, body composition, methane and reproductive performance traits are reported in Table 3. MY was not significantly correlated with PREG or DTC (Table 3), but was significantly phenotypically correlated with CALV, WEAN, P8 and MCWT. The significant correlation between MY and P8 and MCWT provides further evidence, along with the observed divergence between the selection lines for these traits (Table 2), of a significant association between MY and these two traits. The significant correlations of MY with CALV and WEAN is at odds with the lack of divergence between the selection lines for these traits (Table 2), and thus any conclusion about the strength of these associations must await further

analysis of a larger dataset. DTC was significantly (P < 0.0001) correlated with P8 and MCWT (Table 3), with calving later in the season associated with greater P8 fat depth and heavier weight at calf weaning.

Table 3 Phenotypic correlations[a] between selected growth, body composition, methane and reproductive performance traits in heifers from divergent selection lines for methane yield

Trait	Pregnancy Rate	Calving Rate	Days to Calving	Weaning Rate	P8 fat	Cow WT at weaning
Methane Yield	0.006	0.12[*]	-0.09	0.13[*]	-0.46[**]	0.24[**]
P8 fat	-0.01	-0.07	0.13[**]	-0.04	-	-0.18[**]
Cow WT at weaning	-0.47[**]	-0.47[**]	0.23[**]	-0.54[**]	-0.18[**]	-
Joining Age	-0.01	-0.09[*]	0.003	-0.07	0.29[**]	-0.14[**]
Joining WT	-0.01	-0.06	0.006	-0.05	0.22[**]	0.16[**]

[a] Significantly different from 0 at [**] $P \le 0.0001$, and [*] $P \le 0.05$

Selection for MY did not have any impact on pregnancy rate, or weight of the subsequent calf at birth or weaning. However, Low MY females calved later in the calving season and were lighter in weight at calf weaning than High MY females. Calving later in the season could be the result of Low MY females reaching puberty at a later age than High MY females. Donoghue *et al*. (2016b) using a subset of the data in this study reported the regression of MY EBV on age at puberty, with a decrease in MY EBV significantly associated with later age at puberty. A delayed onset of puberty would be expected to be associated with calving later in the season, as observed in Low MY heifers in this study, and a shorter interval between calving and second parity joining, which may explain why Low MY females were lighter in weight at weaning of the first calf.

CONCLUSIONS

It is concluded from this study that selection for Low MY is associated with calving later in the calving season in first-parity heifers. Evidence from a study on a subset of this data would indicate that these results are due to a delay in the onset of puberty in Low MY females. Further investigations are needed to confirm any association between calving and weaning rates and genetic variation in MY, and to determine if any difference in reproductive performance persists through subsequent parities to gain better understanding of impact of selection for methane yield on lifetime reproductive performance.

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