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# ASSOCIATIONS OF RUMEN VOLATILE FATTY ACIDS WITH PHENOTYPIC AND GENETIC VARIATION IN METHANE PRODUCTION TRAITS IN ANGUS CATTLE

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## SUMMARY

Methane emissions from beef cattle contribute to greenhouse gas emissions in the atmosphere and waste gross feed energy consumed by cattle. Screening tests for methane emissions in cattle would be useful in genetic selection programs to reduce emissions. This paper reports results for 136 yearling-age Angus heifers and bulls tested for methane production in respiration chambers, and rumen fluid samples taken 3 hours post-feeding analysed for concentrations of volatile fatty acids (VFAs). A subsample of animals had repeat rumen samples taken 24 hours after feeding. The animals were fed a roughage ration offered at 1.2-times maintenance through testing. Concentrations of major VFAs (acetate, propionate and butyrate) and their proportions in the 3 hours post-feeding sample were strongly associated with methane production (g/d) (correlation coefficients up to 0.62), but less strongly with methane yield and residual methane production (correlation coefficients up to 0.17 and 0.28, respectively). Taking a rumen fluid sample during peak fermentation revealed stronger associations between methane emissions and VFA concentrations than previously reported for samples collected 24 hours after feeding. These relationships open the possibility of using VFA concentrations in rumen samples obtained at peak fermentation as indicator traits for methane emissions. For genetic selection, scrutiny of VFA as a screening test for methane emissions is still warranted.

## INTRODUCTION

Cattle and sheep emit methane, a potent greenhouse gas, as part of the fermentation of feed in their rumen. There exists phenotypic and genetic variation in methane production traits of sufficient magnitude in Angus cattle that breeding for cattle with lower emissions is possible (Bird-Gardiner *et al.* 2015; Donoghue *et al.* 2015). From mechanistic fermentation models, changes in methane production should correspond with changes in the supply of hydrogen from the formation of volatile fatty acids (VFA) during diet substrate fermentation (Ellis *et al.* 2008). However Herd *et al.* (2013) were not able to detect significant associations between methane production rate (L/day) (MP; L/day) and concentrations of VFA in rumen fluid (mmoles/L) collected 24 hours after feeding, and only modest correlations with methane yield (MY = MP per unit feed intake; L/kgDMI) were observed. Those authors concluded that phenotypic associations were too low for VFA concentrations at 24 hours after feeding to be used in screening for high or low methane emitting cattle.

This experiment investigated whether VFA concentrations in rumen fluid collected shortly after feeding, during peak fermentation, were correlated with phenotypic and genetic variation in methane production traits and offer a strategy for screening cattle for methane emissions.

## MATERIALS AND METHODS

A total of 140 animals (62 heifers and 78 bulls) born in the NSW Department of Primary Industries Trangie Angus research herd (Donoghue *et al.* 2015) in 2013 were tested for methane

production as yearlings in 2014 at the University of New England (UNE) methane measurement facility. They were sampled for rumen fluid before being measured for methane production. Animals were moved to UNE in cohorts of approximately 40 animals of the same sex, and kept in group pens for at least 3 days, or until they were tested, in groups of 10. Groups of animals were moved from group feeding pens (last meal offered 24hours earlier) to individual pens, and offered a meal containing their individual daily allowance. Animals were fed lucerne:cereal chaff at 1.2 times maintenance level, based on their body weight record before transport (TWT). Rumen samples were then taken from each animal 3 hours after feed was offered (the same day they were moved to individual pens). This was predicted to be close to peak methane production, based on Deighton *et al.* (2014). A 2-day methane production test was conducted using open circuit respiration chambers (Herd *et al.* 2014) after 2-3days of feeding in the individual pens. For 2 groups of heifers (n=20), a repeat sample was taken following the methane test period, representing approximately 24 hours after feeding, as done previously by Herd *et al.* (2013). Rumen samples, collected using stomach tubing, were preserved by acidification and stored at -18°C. Liquid chromatography was performed on samples to analyse VFA concentrations.

Dry matter intake (DMI; kg/d) was calculated as the average of measured dry matter intake for the two days of methane measurement. Methane production rate was taken as an average over the two days of measurement. Methane yield was calculated as MPR divided by DMI. Residual methane production (RMP; g/d) was calculated as the residual from actual MPR against DMI predicted MPR, from the regression of test data for MPR against DMI, as described in Herd et al. (2014). Phenotypic associations between traits were assessed using Pearson's correlation tests (R Core Team 2014). To assess the associations for VFA traits with genetic variation in methane emissions, correlations were determined with within-herd Estimated Breeding Value (EBV) for MPR, MY and RMP, the latter calculated as described in Donoghue *et al.* (2015). Four animals were removed from the analysis because of large feed refusals.

**Table 1**. **Summary statistics for n=136 yearling Angus bulls and heifers tested for methane production and with rumen fluid samples taken 3 hours post feeding**

| Trait | Average | SD | Maximum | Minimum |
|---|---|---|---|---|
| Pre-test liveweight (TWT; kg) | 390 | 58 | 512 | 270 |
| Dry-matter intake (DMI; kg/d) | 6.2 | 0.8 | 7.8 | 3.5 |
| Methane production rate (MPR; g/d) | 137 | 20 | 180 | 89 |
| Methane yield (MY; g/kg DMI) | 21.9 | 1.2 | 26.1 | 18.2 |
| Residual MPR (RMP; g/d) | 0.0 | 6.6 | 23.1 | -18.0 |
| Acetate (mmoles/L) | 62.3 | 11.5 | 94.2 | 22.0 |
| Propionate (mmoles/L) | 17.8 | 3.8 | 27.4 | 4.0 |
| Iso-butyrate (mmol/L) | 0.24 | 0.10 | 0.43 | 0.02 |
| Butyrate (mmoles/L) | 11.3 | 4.5 | 26.7 | 2.1 |
| Iso-valerate (mmol/L) | 0.94 | 0.33 | 1.75 | 0.20 |
| Valerate (mmol/L) | 1.52 | 0.52 | 3.31 | 0.27 |
| Total VFA (mmoles/L) | 94.1 | 16.1 | 136.8 | 29.3 |
| Acetate% (% total VFA) | 66.3 | 4.9 | 75.0 | 57.2 |
| Propionate (% total VFA)% | 18.9 | 2.4 | 25.6 | 13.7 |
| Butyrate% (% total VFA) | 12.0 | 4.2 | 23.4 | 6.3 |
| EBV_MPR (g/d) | -0.2 | 4.1 | 8.4 | -12.8 |
| EBV_MY (g/kg DMI) | 0.00 | 0.39 | 1.02 | -0.74 |
| EBV_RMP (g/d) | 0.02 | 2.34 | 6.49 | -4.40 |

**RESULTS**

Summary statistics for all animals with 3-hour post-feeding VFA measurements are presented in Table 1. There was substantial variation in methane traits, with MPR strongly influenced by TWT and DMI (Table 2). However, these relationships did not persist for MY or RMP, and there was variation independent of feed intake (CV for MY and RMP; 5.2% and 3.5%). The concentrations and proportions of VFAs (except total VFA) had significant correlations with MPR, but most not with either MY or RMP. Propionate concentration was the only VFA trait which had a significant correlation with MY and iso-valerate was the only VFA with a significant correlation with RMP. Analysis of variance showed that TWT and sex significantly influenced the VFA traits.

Phenotypic variation in the VFA concentrations and ratios were not associated with genetic variation in the 3 methane traits, as indicted by a lack of significant correlation coefficients with the EBV for MPR, MY or RMP (Table 2). The exception was iso-valerate concentration (a minor VFA) which had a significant (P<0.05) correlation with the EBV for MY and RMP (regression coefficients; 0.18 (±0.07) and 0.03 (±0.01)).

**Table 2. Pearson correlations for methane production rate (MPR), methane yield (MY), residual methane production (RMP) and their respective within-herd EBV with pre-test animal weight (TWT), dry-matter intake (DMI) and rumen volatile fatty acid concentrations**

| | MPR (g/d) | MY (g/kg DMI) | RMP (g/d) | EBV_MPR (g/d) | EBV_MY (g/kg DMI) | EBV_RMP (g/d) |
|---|---|---|---|---|---|---|
| TWT (kg) | 0.93*** | 0.13 | -0.01 | 0.35*** | 0.03 | 0.03 |
| DMI (kg/d) | 0.94*** | 0.04 | -0.06 | 0.32*** | 0.02 | 0.02 |
| Acetate (mmoles/L) | -0.26** | -0.13 | -0.03 | -0.07 | -0.06 | -0.06 |
| Propionate (mmoles/L) | 0.19* | -0.17* | -0.13 | -0.00 | -0.04 | -0.04 |
| Iso-butyrate (mmol/L) | -0.55*** | -0.12 | 0.08 | -0.08 | -0.02 | -0.03 |
| Butyrate (mmoles/L) | 0.46*** | 0.05 | -0.07 | -0.03 | -0.07 | -0.07 |
| Iso-valerate (mmol/L) | -0.21* | 0.13 | 0.28*** | 0.13 | 0.22** | 0.21* |
| Valerate (mmol/L) | -0.24** | -0.07 | 0.06 | -0.03 | -0.05 | -0.05 |
| Total VFA (mmoles/L) | -0.03 | -0.12 | -0.06 | -0.06 | -0.07 | -0.07 |
| Acetate% | -0.62*** | -0.05 | 0.06 | 0.00 | 0.00 | 0.01 |
| Propionate% | 0.39*** | -0.13 | -0.12 | 0.02 | 0.02 | 0.02 |
| Butyrate % | 0.57*** | 0.12 | -0.04 | -0.04 | -0.04 | -0.04 |

*P<0.05; **P<0.01; ***P<0.001.

Linear models to predict methane from multiple VFAs indicated significant (P<0.05) models utilising the major VFAs and Isovalerate, with or without Iso-butyrate, and some interactions, explain variation in methane traits and their EBVs. These models were stronger for methane traits (P<0.001 and $R^2$=0.52,0.28 and 0.23 for MPR, MY and RMP), and weaker for EBVs (P<0.05, $R^2$=0.14 for EBV_MPR and P<0.01, $R^2$=0.19 and 0.17 for EBV_MY and EBV_RMP).

For the 19 animals with repeated rumen fluid samples and methane data, VFA concentrations were generally higher in samples taken at 3 hours compared to those taken 24 hours after feeding (eg. Total VFA 96.9mmoles/L at 3hr and 61.4 mmoles/L at 24hr). Correlations between the 3-hour and 24-hour sample values were generally low or negative, except for propionate concentration at -0.47 (only significant correlation, P<0.05), with low correlations for their proportions.

In general, the magnitude of the correlations for the major VFAs with MPR were higher for the 3-hour sample (0.26 to 0.13) than for the 24-hour sample (0.05 to 0.07). Correlations at 3-hours and 24-hours with MY and RMP were in different directions. However, the only statistically-

significant (P<0.05) correlation in this data set was acetate proportion at 24 hours after feeding with MY (r=0.46), with none of the correlations with 3hr samples reaching significance.

## DISCUSSION

Comparing the results from the repeated samples taken 24 hours after feeding during this study with the results from Herd *et al.* (2013), all taken 24hours after feeding, the concentrations of the major fatty acids fall within one standard deviation of the mean from the previous study. In common with Herd *et al*. (2013), the associations between total VFA, concentrations of the three most abundant VFA, and their molar proportions in rumen fluid samples collected 24 hours after feeding, with MPR, were weak. With MY the relationships were in the same direction as reported by Herd *et al*. (2013), although mostly non-significant in the present experiment, presumably due to the small sample size. Herd *et al*. (2013) concluded that the strength of the phenotypic associations between VFA concentrations in rumen fluid taken 24 hours after feeding with methane emissions to be too low for an accurate screening test for high or low emitting cattle.

Taking a rumen fluid sample during peak fermentation revealed stronger associations between methane production and VFA concentrations than reported by Herd *et al*. (2013) for samples collected 24 hours after feeding. These stronger relationships open the possibility of using VFA concentrations in rumen samples obtained at peak fermentation as indicator traits for variation in MP if feed intake cannot be measured. The significant relationships with MY reported by Herd *et al.* (2013) were not replicated in the VFA concentration in 24-hour rumen samples collected in this study. For genetic selection, more careful scrutiny of VFA as markers for methane emissions is still warranted.

Examining the relationships between the new EBVs for methane traits and VFA concentrations, the only VFA with a significant relationship with methane trait EBVs was Iso-valerate concentration, a minor VFA, with MY and RMP and these relationships were still relatively weak. Iso-valerate is associated with fibre digestion (Liu *et al.* 2009), and differences in iso-valerate concentrations have been related to Net Feed Intake (Hernandez-Sanabria *et al.* 2010). This relationship is worth further investigation to understand how rumen fermentation changes as animals are genetically selected for increased or decreased methane emissions.

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