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Enteric methane emissions in response to ruminal inoculation of *Propionibacterium* strains in beef cattle fed a mixed diet

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Abstract. The objective of this study was to test the efficacy of *Propionibacterium* strains to mitigate enteric methane (CH_4) emissions in beef heifers fed a mixed diet. An experiment was conducted with 16 ruminally cannulated beef heifers fed a basal diet consisting of 60 : 40 barley silage : barley grain (DM basis). Treatments included: (1) Control, (2) *Propionibacterium freudenreichii* T114, (3) *P. thoenii* T159, and (4) *P. freudenreichii* T54. Strains (1×10^{11} colony forming units) were administered daily directly into the rumen before feeding. No treatment effects were observed for DM intake ($P = 0.90$), mean ruminal pH ($P = 0.50$) and total volatile fatty acids ($P = 0.44$). However, compared with the Control, proportions of individual volatile fatty acids changed with acetate being less with *Propionibacterium* T159 ($P = 0.02$), whereas ruminal isobutyrate ($P < 0.01$) and acetate : propionate ratio ($P = 0.04$) were greater with *Propionibacterium* T114. Total daily enteric CH_4 production averaged 188 g/day and was not affected by *Propionbacterium* strains ($P = 0.51$). Methane yield averaged 22 g/kg of DMI intake and tended to be greater with *Propionibacterium* strains ($P = 0.08$). The relative abundance of total *Propionibacteria* was greater with the inoculation of *Propionibacterium* T159 relative to the Control heifers ($P = 0.04$). In conclusion, inoculation of *Propionibacterium* T159 decreased ruminal acetate proportion and *Propionibacterium* T114 increased acetate : propionate ratio. However, inoculated strains failed to lower total CH_4 emissions possibly due to the inability of *Propionibacterium* strains to elevate ruminal propionate concentrations.

Additional keywords: beef, methane, *Propionibacterium*.

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Introduction

Some strains of *Propionibacteria* are natural propionate producers that inhabit the rumen and comprise 1.4% to 4.3% of the total microbial population (Mead and Jones 1981). The development of *Propionibacterium* strains as direct-fed microbials could offer an effective means of increasing ruminal propionate production and reducing enteric methane (CH_4) emissions from cattle fed forage-based diets (Jeyanathan *et al*. 2014). Previous studies explored the potential to use *Propionibacterium* strains to mitigate CH_4 emissions in beef cattle fed high-forage (Vyas *et al*. 2014*a*) and high-grain diets (Vyas *et al*. 2014*b*). However, no effects were observed on total CH_4 emissions due to low persistency of the inoculated strains. Contrary to the previous *in vivo* studies (Vyas *et al.* 2014*a*, 2014*b*), *in vitro* batch culture studies with *Propionibacterium* strains showed promising results with significant reduction in CH_4 production using both high-forage and high-grain diets (Alazzeh *et al*. 2013). The discrepancy between studies might be related to different strains of *Propionibacteria* used in the *in vitro* batch culture experiment compared with the *in vivo* study. The efficacy of *Propionibacterium* strains identified using an *in vitro* batch culture experiment as having CH_4 mitigation potential (Alazzeh *et al*. 2013) needs to be validated *in vivo* before such a strategy can be recommended for lowering CH_4 emissions from beef cattle. Hence, the primary objective of this study was to confirm *in vivo* the efficacy of *Propionibacterium* strains previously identified *in vitro* as having the potential to mitigate CH_4 emissions in beef cattle.

Materials and methods

Animal, diets and experimental design

The protocol for the study was approved by Lethbridge Research Centre Animal Care Committee before the experiment began and animals were cared for according to the guidelines of the Canadian Council on Animal Care (1997). Sixteen ruminally cannulated crossbred beef heifers were used in this study. The heifers were grouped on the basis of pre-experimental bodyweight (mean ± s.d.: Group 1 = 602 ± 31 kg, Group 2 = 570 ± 60 kg, Group 3 = 590 ± 50 kg and

Group 4 = 620 ± 27 kg). Dietary treatments included: (1) Control, (2) *Propionibacterium freudenreichii* T114, (3) *P. thoenii* T159, and (4) *P. freudenreichii* T54. Strains were grown daily in sodium lactate broth under anaerobic conditions according to the method described earlier (Alazzeh *et al*. 2013) and were administered daily (1×10^{11} colony forming units) at the time of feeding directly into the rumen. Treatments were randomly allotted within each group. All heifers were fed the basal diet (60 : 40 forage to concentrate [(dry matter (DM) basis); Table 1] formulated to provide adequate metabolisable energy and protein for 600 kg growing beef cattle with an average daily gain of 1 kg/day (NRC 2000). Heifers were fed for *ad libitum* intake once daily at 1300 hours, housed in a ventilated tie-stall barn, and exercised daily in an open dry lot. Diets were supplemented with melengesterol acetate (1.3 mg/head.day; MGA-100 premix, Pfizer Animal Health, Pfizer Canada Inc., Kirkland, QC, Canada) to suppress oestrus and prevent ovulation in the beef heifers.

Table 1. Ingredient and chemical composition of the total mixed ration and the melengestrol acetate (MGA) supplement

Item	% DM
Ingredient	
Barley silage[A]	60.0
Barley grain, dry rolled[B]	32.5
Supplement[C]	5.0
Canola meal	2.060
Canola oil	0.027
Barley, ground	1.760
Limestone	0.150
Salt	0.025
Urea	0.200
Molasses	0.750
Vitamin E (500 000 IU/kg)	0.003
Feedlot premix[D]	0.025
MGA[E]	2.50
Barley grain, ground	2.430
Molasses, dried	0.055
MGA-100 premix	0.013
Flavouring cattle	0.002
Chemical composition	
DM (%)	56.3 ± 2.40
Organic matter (% of DM)	90.5 ± 0.65
Crude protein (% of DM)	12.1 ± 0.80
Neutral detergent fibre (% of DM)	40.7 ± 3.96
Acid detergent fibre (% of DM)	21.3 ± 4.22

[A]Composition (DM basis): 33.2 ± 3.06 DM, 88.9 ± 4.10 organic matter, 11.1 ± 1.78 crude protein, 54.1 ± 2.43 neutral detergent fibre and 31.9 ± 1.33 acid detergent fibre.

[B]Composition (DM basis): 90.5 ± 0.40 DM, 97.2 ± 0.32 organic matter, 13.03 ± 1.49 crude protein, 20.8 ± 0.98 neutral detergent fibre, 5.14 ± 0.90 acid detergent fibre.

[C]Composition (DM basis): 94.0 ± 0.21 DM, 62.2 ± 4.0 organic matter, 18.5 ± 0.32 crude protein, 22.0 ± 3.73 neutral detergent fibre, 6.07 ± 0.45 acid detergent fibre.

[D]Feedlot premix provided an additional 14 g/kg Ca, 103 mg/kg Zn, 26 mg/kg Cu, 47 mg/kg Mn, 1 mg/kg I, 0.50 mg/kg Se, 0.33 mg/kg Co, 17187 IU/kg vitamin A, 859 IU/kg vitamin D_3 and 24 IU/kg vitamin E of the diet DM.

[E]Melengestrol acetate; Composition (DM basis): 91.6 ± 1.21 DM, 96.2 ± 0.70 organic matter, 12.5 ± 1.32 crude protein, 15.9 ± 3.23 neutral detergent fibre, 6.57 ± 1.45 acid detergent fibre.

Data and sample collection

During the experiment, Days 1 to Day 14 were used to adapt heifers to their treatments. Ruminal contents were collected on Day 15 and Day 18 at 0, 3, 6 and 9 h post feeding. Ruminal pH was measured continuously from Day 15 to Day 21, and enteric CH_4 emissions were measured from Day 19 to Day 21. Daily intakes and orts of the diets for individual heifers were recorded. Diets and orts were sampled daily during days of CH_4 measurement and were pooled for each animal at the end of the period of CH_4 measurement. Dietary ingredients were sampled once weekly and analysed for DM by drying at 55°C for 72 h. Ingredients and total mixed ration samples were stored at −20°C until analysed.

Methane emissions were measured from individual heifers for 3 days using environmental chambers as described earlier (Beauchemin and McGinn 2006). Briefly, chambers were calibrated before and after each period by sequentially releasing 0, 0.2, and 0.4 L/min of CH_4 (Praxair Canada Inc., Mississauga, ON, Canada) separately into each empty chamber using a mass-flow meter (Omega Engineering, Stamford, CT, USA). A 3-point regression was developed by plotting actual against calculated CH_4 emission ($R^2 = 0.99$). The slopes of these best fit linear relationships were used to correct for between-chamber variability. Conditions of air circulation and sampling procedures were as described by Avila-Stagno *et al*. (2013).

To determine the effect of *Propionibacteria* on ruminal pH, daily pH profiles were measured starting at feeding on Day 15 using an indwelling pH data acquisition system (LRC pH dataloggers, Dascor, Escondido, CA, USA) that was retained in the rumen for 7 days (includes the period of CH_4 measurement). The system was standardised using pH 4 and 7 buffers before insertion on the first day and then upon removal on the last day as described earlier (Penner *et al*. 2006). On Days 15 and 18, at 0, 3, 6, and 9 h post feeding, ruminal contents were sampled from four different sites (cranial, caudal, ventral, and dorsal sacs), composited and strained through a double layer of polyester monofilament fabric (Pecap 7–1180/59, mesh opening 1180 µm, Tetko Inc., Scarborough, ON, Canada). Two samples of filtered ruminal fluid (5 mL) were preserved by adding 1 mL of 25% (wt/vol) phosphoric acid for volatile fatty acids (VFA) and lactate determination, and 1 mL of 1% (wt/vol) sulfuric acid for ammonia-N (NH_3-N) determination. The samples were stored at −20°C until analysed.

Rumen samples collected at 0, 3, and 9 h were processed for microbial analysis, separately for each heifer. Microbial pellet was extracted based on a method described earlier (Vyas *et al*. 2014*a*). Quantitative real-time PCR assays were performed with a 7900 HT Fast Real-time PCR system (Applied Biosystems, Foster City, CA, USA) using POWER SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), forward and reverse primers (500 nM of each primer/reaction), and ~20 ng of template DNA in a final volume of 25 µL per reaction. The primers for universal bacteria (forward primer: 5′-TCCTAC GGGAGGCAGCAGT-3′; reverse primer: 5′-GGACTACCA GGGTATCTAATCCTGTT-3′; Nadkarni *et al*. 2002) and total

Propionibacteria (forward primer: 5′-RGTGGCGAAGGCGGT TCTCTGGA-3′; reverse primer: 5′-TGRGGTCGAGTTGCAG ACCCCAAT-3′; Rossi *et al*. 1999) were used. Amplifications were performed under the conditions described earlier (Vyas *et al*. 2014*a*). The relative population size of total *Propionibacteria* was determined as the ratio of the amplification of total *Propionibacteria* 16S rRNA to the amplification of the universal bacteria. PCR efficiency was calculated using the formula E = [10(–1/slope) – 1]. The slopes ranged from –3.37 to –3.40 for total bacterial primer and –3.29 to –3.30 for total *Propionibacteria*.

Laboratory analyses

Dry matter for all samples was determined by oven drying at 55°C for 72 h. Dried samples were ground in a Wiley mill (A. H. Thomas, Philadelphia, PA, USA) through a 1-mm screen. Analytical DM content of the ground sample was determined by drying at 135°C for 2 h (method 930.15; AOAC 2005), followed by hot weighing. The organic matter content was calculated as the difference between 100 and the percentage ash (method 942.05; AOAC 2005). The neutral detergent fibre and acid detergent fibre contents were determined according to Van Soest *et al*. (1991) with heat stable amylase and sodium sulfite used in the neutral detergent fibre procedure. Samples were ground using a ball mill (Mixer Mill MM2000, Tetsch, Haan, Germany) for the determination of crude protein. Total N was quantified by flash combustion and thermal conductivity detection (Carlo Erba Instuments, Milan, Italy). Ruminal VFA, lactate and NH_3-N concentration were quantified as described earlier (Vyas *et al*. 2014*a*). Gross energy concentration was determined using a bomb calorimeter (model E2k, CAL2k, Johannesburg, South Africa).

Statistical analyses

The data were analysed using the MIXED procedure of SAS with heifer as the experimental unit. For data that were collected serially [DM intake (DMI), CH_4, and ruminal fermentation] the model included the fixed effect of treatment, sampling time and their interaction, with sampling time considered as a REPEATED effect in the model. Group was used in the RANDOM statement. Variance components were estimated by the restricted maximum likelihood method. Kenward–Roger's option was used in the MODEL statement to estimate denominator degrees of freedom. Time-series covariance structure was modelled using the options of autoregressive order-one, compound symmetry, and unstructured order-one. Best time-series covariance structure for each variable was selected based on lowest Akaike and Bayesian information criteria. CONTRAST statement was used to evaluate differences between means of Control and *Propionibacterium* treatments. Data are presented as least-squares means ± standard error of the means. Statistical significance was declared at $P \leq 0.05$ and trends are discussed at $P \leq 0.10$.

Results

No treatment effects were observed on DMI ($P = 0.90$; Table 2) or ruminal pH variables. Likewise, total VFA production was similar across all treatments ($P = 0.44$; Table 3). Ruminal acetate proportion was reduced with *Propionibacterium* T159 ($P = 0.02$) whereas no effects were observed with other strains. Correspondingly, no treatment effects were observed on proportion of ruminal propionate ($P = 0.12$). The proportion of ruminal isobutyrate ($P < 0.01$) and acetate : propionate ratio was increased ($P = 0.04$) with *Propionibacterium* T114. Ruminal NH_3-N concentration was similar for all the treatments ($P = 0.79$).

No treatment differences were observed for DMI on the days of CH_4 measurement in chambers ($P = 0.65$; Table 4). However, DMI in chambers was reduced by 5–21% compared with DMI measured during the metabolism experiment, with a greater decline observed in the animals receiving *Propionibacterium* strains, primarily *Propionibacterium* T159. Total enteric CH_4 production was not affected by treatments and averaged 188 g/day ($P = 0.51$). Methane yield adjusted for DMI ($P = 0.19$) and gross energy intake ($P = 0.17$) were similar across all the treatments. However, contrary to our hypothesis, CH_4 yield adjusted for DMI and gross energy intake tended to increase when means were compared between Control and *Propionibacterium* treatments ($P = 0.08$). The numerical differences in total CH_4 emissions with the inoculation of *Propionibacterium* strains were driven by changes observed during the initial 0–10 h post feeding (Fig. 1).

Inoculation of *Propionibacterium* T159 increased the relative abundance of total *Propionibacteria* probably due to

Table 2. Dry matter intake (DMI) and ruminal pH for beef heifers fed a mixed diet supplemented with Control or *Propionibacterium* strains T114, T159 or T54[A]

Variable	Treatment				s.e.m.	Effect (*P*-value)			
	Control	T114	T159	T54		Treatment	Day	Treatment × Day	Control vs *Propionibacterium*
No. of observations	4	4	4	4	–	–	–	–	–
DMI (kg/day)	10.44	9.42	9.86	9.83	1.24	0.90	0.06	0.94	0.53
					Ruminal pH				
Minimum pH	5.85	5.58	5.88	5.90	0.14	0.25	<0.01	0.44	0.66
Mean pH	6.37	6.23	6.45	6.47	0.11	0.50	<0.01	0.08	0.94
Maximum pH	6.87	6.90	6.95	6.96	0.08	0.86	<0.01	0.34	0.51

[A]*Propionibacterium* strains T114, T159 and T54 (1×10^{11} colony forming units) were administered in the rumen daily at the time of feeding.

Table 3. Ruminal fermentation characteristics of beef heifers fed a mixed diet supplemented with Control or *Propionibacterium* strains T114, T159 or T54[A]

a, b values within a row with different letters differ ($P \leq 0.05$)

Variable	Treatment				s.e.m.	Effect (*P*-value)			
	Control	T114	T159	T54		Treatment	Hour[B]	Treatment × Hour	Control vs *Propionibacterium*
No. of observations	4	4	4	4	–	–	–	–	–
Total volatile fatty acids (VFA, mM)	115.1	108.0	114.3	108.4	5.8	0.44	<0.01	0.70	0.30
Individual VFA (mol/100 mol)									
Acetate (A)	63.8a	65.2a	61.1b	63.3a	0.8	0.02	<0.01	0.35	0.52
Propionate (P)	19.0	16.8	20.0	18.3	0.9	0.12	0.04	0.98	0.53
Isobutyrate	1.00b	1.18a	1.01b	1.06b	0.04	<0.01	<0.01	0.93	0.03
Butyrate	11.5	11.9	13.7	12.8	1.0	0.39	<0.01	0.10	0.24
Valerate	1.69	1.73	1.69	1.75	0.06	0.85	<0.01	0.09	0.69
Isovalerate	2.07	2.05	1.70	1.81	0.17	0.39	0.07	0.98	0.30
Caproate	0.91	1.10	0.73	0.92	0.09	0.12	<0.01	0.50	0.99
Lactate (mM)	ND[C]	ND	ND	ND	–	–	–	–	–
A : P ratio	3.40b	3.95a	3.12b	3.48ab	0.18	0.04	<0.01	0.95	0.55
Ammonia-N (mM)	4.30	4.49	4.01	4.45	0.54	0.79	<0.01	0.09	0.97

[A]*Propionibacterium* strains T114, T159 and T54 (1×10^{11} colony forming units) were administered in the rumen daily at the time of feeding.
[B]Rumen samples were taken on Days 15 and 18, at 0, 3, 6, and 9 h post feeding.
[C]Not detected.

Table 4. Enteric methane (CH_4) emissions from beef heifers housed in chambers and fed a mixed diet supplemented with Control or *Propionibacterium* strains T114, T159 or T54[A]

DMI, dry matter intake; GE, gross energy

Variable	Treatment				s.e.m.	Effect (*P*-value)			
	Control	T114	T159	T54		Treatment	Day	Treatment × Day	Control vs *Propionibacterium*
No. of observations	4	4	4	4	–	–	–	–	–
DMI (kg/day)	9.30	8.87	7.78	8.60	0.86	0.65	<0.01	0.68	0.38
CH_4 (g/animal.day)	182.8	210.0	172.3	186.4	17.6	0.51	<0.01	0.52	0.75
CH_4 yield									
CH_4 (g/kg of DMI)[B]	20.0	24.1	22.7	21.6	1.5	0.19	0.11	0.93	0.08
Percentage of GE intake	6.38	7.76	7.22	6.92	0.43	0.17	0.11	0.93	0.08

[A]*Propionibacterium* strains T114, T159 and T54 (1×10^{11} colony forming units) were administered in the rumen daily at the time of feeding.
[B]Enteric CH_4 production (g of CH_4/animal per day) expressed relative to DMI determined on the days of CH_4 measurement.

the greater prevalence of the respective strain ($P = 0.04$; Fig. 2). However, no effects were observed on the relative abundance of total *Propionibacteria* with the inoculation of other strains. Relative abundance of total *Propionibacteria* was not affected by sampling time ($P = 0.27$).

Discussion

Strategies to mitigate CH_4 emissions in cattle fed a mixed diet are desirable as emissions are higher from feedlot cattle during the growing, as compared with the finishing, phase of beef production (Beauchemin and McGinn 2005). Recently, the role of *Propionibacterium* species in reducing CH_4 emissions was explored in beef cattle fed a high-forage diet (Vyas *et al.* 2014*a*) and a high-grain diet (Vyas *et al.* 2014*b*); however, inoculated strains failed to increase ruminal propionate proportion and mitigate total CH_4 emissions. It is possible that the lack of effect of *Propionibacterium* species in those studies was due to the strains selected; thus, the present study examined additional *Propionibacterium* strains. The *Propionibacterium* strains used in the present study were previously screened for their CH_4 mitigation potential *in vitro* using both forage- and grain-based diets (Alazzeh *et al.* 2013), unlike in the studies of Vyas *et al.* (2014*a*, 2014*b*). Animals used in the present study had no previous exposure to *Propionibacterium* strains thereby ruling out the possibility of any carry-over effects that might have confounded results in the present study.

The present *in vivo* study showed no significant treatment effects on total CH_4 production for any of the strains used, in contrast to observations from a previous *in vitro* study (Alazzeh *et al.* 2013). Moreover, total enteric CH_4 emissions corrected for DM and gross energy intake tended to be greater with the inoculation of *Propionibacterium* strains. The results observed in the present study are contrary to the suppression of CH_4 yield

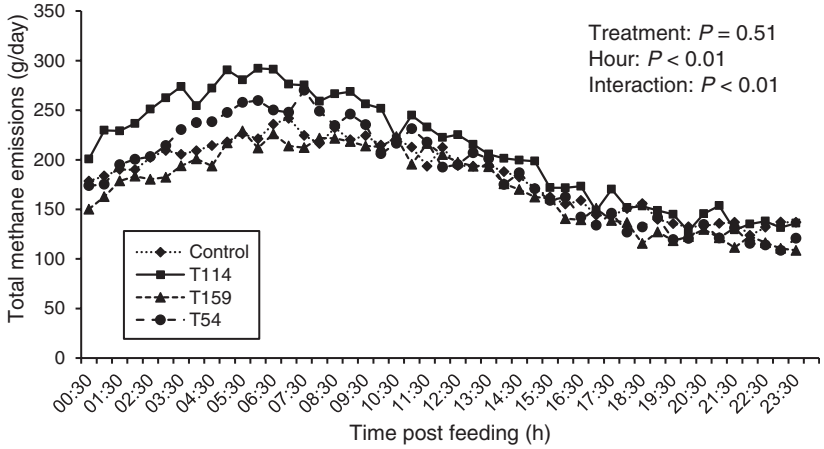

Fig. 1. Total methane emissions post feeding in beef heifers fed a mixed diet supplemented with Control or *Propionibacterium* strains T114, T159 or T54. *Propionibacterium* strains T114, T159 and T54 (1×10^{11} colony forming units) were administered in the rumen daily at the time of feeding.

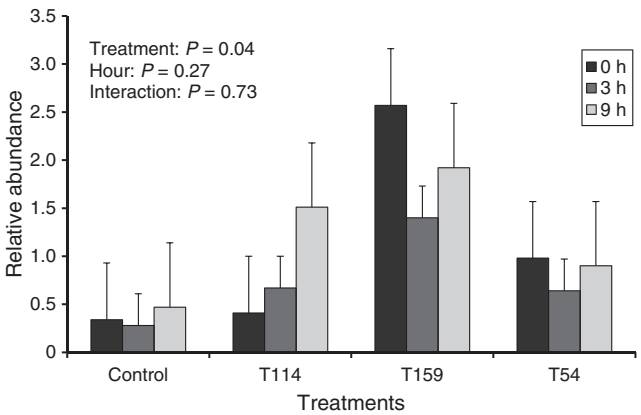

Fig. 2. Relative abundance[A] of total ruminal Propionibacteria at 0, 3 and 9 h post feeding in beef heifers fed a mixed diet supplemented with Control or *Propionibacterium* strains T114, T159 or T54[B,C]. [A]Relative abundance was determined as the ratio of copies of total *Propionibacteria* to copies of total bacteria and expressed as percentage. [B]Treatment effect: Control, 0.37[a]; T114, 0.87[a]; T159, 1.96[b]; T54, 0.84[a]; s.e.m. 0.36 ([a,b] Values with different letters differ; $P \leq 0.05$). [C]*Propionibacterium* strains T114, T159 and T54 (1×10^{11} colony forming units) were administered in the rumen daily at the time of feeding.

observed earlier with *Propionibacterium* strains inoculated under similar dietary conditions (Vyas *et al*. 2014*a*). The inconsistency between results might be attributed to the use of different strains or species of *Propionibacterium* across the two studies and their differential effects on DMI under stressful conditions when animals were in chambers. For some unknown reason, in the present study the drop in the DMI in chambers was more prominent for animals inoculated with *Propionibacterium* strains than Control cattle. Given that intake affects ruminal passage rate of digesta (Sniffen *et al*. 1992), reduced intake with *Propionibacterium* strains might have increased retention time of substrates in the rumen resulting in greater fermentation and thereby greater CH_4 emissions. A similar inverse relationship between CH_4 production and ruminal passage rates was observed previously where CH_4 production was decreased by 29% with 63% increase in the fractional passage rate of particulate matter in steers (Okine *et al*. 1989).

The absence of treatment effects on total CH_4 emissions observed in a previous study by Vyas *et al*. (2014*a*) was attributed to the lack of survival and persistence of inoculated *Propionibacterium* strains as the abundance of the inoculated bacteria returned to pre-treatment levels within 9 h post inoculation. In contrast, in the present study, the relative abundance of total *Propionibacteria* was greater with the inoculation of *Propionibacterium* T159, with greater levels of abundance at every time point post inoculation, relative to the Control. Discrepancy between studies might be due to the use of different *Propionibacterium* strains and better adaptability of *Propionibacterium* T159 to ruminal conditions in animals fed mixed diets. The lack of survival and persistence of *Propionibacterium* strains in the rumen observed previously (Vyas *et al*. 2014*a*) was attributed to absence of ruminal lactate, a preferred substrate for the growth of *Propionibacterium* spp. Although ruminal lactate was not detectable in the present study, better persistence of *Propionibacterium* T159 could have been due to utilisation of alternative substrates including glucose as well as amino acids to produce propionate and acetate (Piveteau 1999). The presence of metabolically active *Propionibacterium* T159 might have accounted for the reduced molar proportion of ruminal acetate; however, lack of significant effect on total CH_4 emissions might be attributed to the inefficacy of *Propionibacterium* T159 to increase ruminal propionate.

Contrary to the effects on VFA profile observed with *Propionibacterium* T159, inoculation of *Propionibacterium* T114 increased acetate : propionate ratio. The relative abundance of total *Propionibacteria* in the rumen of heifers inoculated with *Propionibacterium* T114 suggested a lack of persistence of the inoculated strain making it difficult to explain the induced changes in ruminal VFA profile. It is possible that the relative abundance of total *Propionibacteria* with the inoculation

of *Propionibacterium* T114 was below the detection limit, yet sufficient enough to influence and induce corresponding changes in ruminal VFA profile.

The lack of response on ruminal fermentation and CH_4 emissions with the inoculation of *Propionibacterium* T54 might be attributed to the absence of metabolically active *Propionibacterium* T54 given that there was no significant increase in relative abundance of total *Propionibacteria* post inoculation of the respective strain. The effects on ruminal fermentation and CH_4 emissions with *Propionibacterium* T114 and T54 are contrary to the *in vitro* results observed earlier (Alazzeh *et al*. 2013). The discrepancy between studies could be attributed to the use of different methods for studying ruminal fermentation. The *in vivo* method used in the present experiment is more representative of the biological system as compared with the *in vitro* batch culture experiment used earlier (Alazzeh *et al*. 2013).

It should also be acknowledged that failure to detect numerical differences observed on total CH_4 emissions and CH_4 yield could also be attributed to the lack of statistical power of the experiment. Hence, results observed from the present study might definitively dismiss the role of *Propionibacterium* strains on mitigating CH_4 emissions; however, future studies with greater replication are required to validate the results observed in this study.

In conclusion, *Propionibacterium* strains induced changes in ruminal VFA profile but failed to elicit significant treatment differences in total CH_4 emissions probably due to the inability of *Propionibacterium* strains to significantly alter ruminal propionate concentrations. When results of this study are examined together with previous *in vivo* studies, it appears that supplementing cattle diets with *Propionibacterium* has limited potential to mitigate enteric CH_4 emissions.

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