Acta Agriculturae Scandinavica, Section A — Animal Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/saga20

Ruminal survival of *Propionibacterium thoenii* T159 in dairy cows at high feed intake

Jikun Chen, Helge Holo, Angela Schwarm & Odd Magne Harstad

To cite this article: Jikun Chen, Helge Holo, Angela Schwarm & Odd Magne Harstad (2020) Ruminal survival of *Propionibacterium thoenii* T159 in dairy cows at high feed intake, Acta Agriculturae Scandinavica, Section A — Animal Science, 69:3, 189-191, DOI: 10.1080/09064702.2020.1769719

To link to this article: https://doi.org/10.1080/09064702.2020.1769719

Published online: 01 Jun 2020.

ACTA AGRICULTURAE SCANDINAVICA, SECTION A — ANIMAL SCIENCE
2020, VOL. 69, NO. 3, 189–191
https://doi.org/10.1080/09064702.2020.1769719

Taylor & Francis
Taylor & Francis Group

BRIEF REPORT

Ruminal survival of *Propionibacterium thoenii* T159 in dairy cows at high feed intake

Jikun Chen[a,b], Helge Holo [b,c], Angela Schwarm [a] and Odd Magne Harstad[a]

[a]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway; [b]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway; [c]Tine SA, Oslo, Norway

ABSTRACT

Propionibacterium thoenii strain T159 (5×10^{11} CFU) were administered daily in the rumen of four Norwegian Red dairy cows. Total *Propionibacterium* in the rumen were substantially increased during and after the treatment with T159 relative to the background. Strain T159 was able to persist for at least five days in the rumen of dairy cows at high dry matter intake (3.9% of body weight).

ARTICLE HISTORY
Received 19 March 2020
Accepted 10 May 2020

KEYWORDS
Propionic acid bacteria; direct-fed microorganisms; Norwegian Red; ruminant; ruminal persistence

Introduction

Propionic acid bacteria (PAB) produce propionic acid as a by-product of (feed) fermentation. PAB isolated from dairy products have been applied as direct-fed microorganisms (DFM) for ruminants to increase ruminal propionate proportions (Stein et al., 2006; Weiss et al., 2008). DFM can cause a shift in rumen fermentation from acetate to propionate synthesis. This glucogenic volatile fatty acid can improve the energy available to the ruminant and thus its production performance (Stein et al., 2006; Vyas et al. 2016). In addition, DFM also have the potential to reduce H_2 generation and thus reduce enteric methane production. *Propionibacterium acidipropionici* strain P169 and *P. thoenii* strain T159 are among the most promising DFM in terms of ruminal survival and rumen fermentation effects. In beef heifers it was found that administered strain P169 (Vyas et al., 2014) and T159 (Vyas et al., 2016) could persist for 9 h at elevated levels in the rumen. However, ruminal DFM detectability beyond this time point was not studied. Establishment and ruminal survival of PAB is likely influenced by feeding level. The objective of this work was to determine the ability of *Propionibacterium thoenii* strain T159 to persist for several days in the rumen of dairy cows at high feed intake.

Materials and methods

The experiment was authorized by the Norwegian Animal Research Authority. Four multiparous, rumen-cannulated Norwegian Red cows were initially 35 ± 18 days in milk (mean ± SD), had a body weight of 558 ± 29 kg and yielded 32.9 ± 7.2 kg milk daily. The cows were housed in tie-stall, had free access to water and were milked at 0730 and 1930 h. Cows were allowed <1% feed refusal and offered daily 8.6 kg dry matter (DM) of concentrate (in g/kg DM; crude protein, 223, NDF, 180, starch, 337) and 13 kg DM of grass silage (in g/kg DM; NDF, 539, CP, 147). The feed was divided into three equal portions and provided at 07.00, 14.00 and 21.00 h. The experiment lasted 15 days and consisted of three sampling periods: pre-treatment (control), treatment and post treatment. Samples of rumen content were collected through the rumen cannula. Control samples were collected at days −2, −1 and 0. PAB were administered through the rumen cannula at days 0–7 as detailed below. At 3 and 24 h after PAB treatment, samples were collected at days 1–8, and post treatment samples at days 9–12.

Propionibacterium thoenii T159 was grown anaerobically at 30°C for 48 h in sodium lactate broth (SLB) liquid medium (Malik et al., 1968). Five hundred mL of T159 liquid culture (1×10^9 CFU/mL) was administered daily at 13.50 h to each cow through the rumen cannula. On the first day of treatment (day 0), all cows were administered with an initial dose of 1.0 L culture. Rumen content (mixture of fluid and solid material) was collected through the rumen cannula at 13.40 h during the control period and at 13.40 and 17.00 h

CONTACT Helge Holo helge.holo@nmbu.no

subsequently. A total of one liter of rumen content was collected at 5 depths from the ventral to the dorsal rumen. The sample was thoroughly mixed, and 50 mL of rumen content was transferred into plastic tubes and immediately frozen at −18°C.

The levels of rumen *Propionibacterium* spp. were analyzed by using polymerase chain reaction (PCR) with primers targeting their 16S rRNA gene. The thawed samples were processed in a Stomacher 400 Circulator (Seward, UK) to detach microbes from solid rumen contents. After filtration with 2 layers of cheese cloth, 100 µL aliquots were transferred to 1.5 mL centrifuge tubes and subjected to microwave treatment at 800 W for 1 min. The DNA was isolated using a NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel) according to the manufacturer's instructions with the following modifications: The microwave treated samples were mixed with 200 µL of the chaotropic salt solution. The mixtures were left at room temperature for 1 min, then centrifuged at 10,000 × g for 1 min and the supernatants were applied to silica filter columns. After the washing steps DNA was eluted in 30 µL. Serial dilutions of the DNA were made in water and served as template in PCR with OneTaq polymerase (New England Biolabs, Massachusetts, USA) using primers PAB1 (5′ – TAG GGT GCG AGC GTT GTC CG – 3′) and PAB4 (5′ – GAA CCG CCT TCG CCA CTG GT – 3′) at 0.2 µM, 1 µL DNA and 0.25 µL polymerase in 50 µL reactions. The following protocol was used: 5 min initial denaturation at 94°C followed by 35 cycles of denaturation (15 s at 94°C), annealing (30 s at 68°C), and extension (5 min at 68°C). PCR products were analyzed by electrophoresis of 10 µL aliquots in 2% agarose gels with 0.004% peqGREENTM (VWR International, Lutterworth, UK) and visualized by UV light. The level of PAB was calculated from the highest dilution of template DNA giving a detectable PCR product and related to DNA from a culture of T159 of known cell density. Microwave treatment has been shown to release DNA from various bacteria (Bollet et al., 1991), but our microwave treated rumen samples contained unknown substances inhibiting the PCR reaction and no PCR product was obtained even from the samples taken after administration of T159. We therefore introduced a simple cleanup procedure using silica. A centrifugation step was included to remove insoluble material. The sensitivity of this analysis was compared by colony counting on SLB agar. The results showed that using the DNA isolation procedure described we were able to detect 10^4 CFU/mL (corresponding to 30 CFU per reaction) of strain T159 by PCR.

Means of PAB concentrations were compared between treatment and post treatment period by Student's paired *t*-tests using Minitab (19, Minitab Inc., PA, USA). Significance level was set to $P < .05$.

Results and discussion

Before administration of T159, the PCR analysis indicated a background level of PAB of 10^4 CFU/mL in the rumen content control samples (day −2, −1, 0) of all cows, which is consistent with the results from Davison (1998). This low background allowed us to investigate the persistence of the administered T159. In the studies in beef heifers (Vyas et al., 2014, 2016) and dairy cows (Jeyanathan et al., 2019), the background PAB numbers were much higher and could complicate the determination of PAB persistence. The addition of T159 into the rumen caused a strong increase in the concentration of PAB in ruminal content (Figure 1). The increase was about 2 logs, close to the value expected from the dose given, and was observed already after the first administration. We did not detect any difference in PAB numbers between samples taken at 3 and 24 h after T159 administration (results not shown). Daily administration of T159 for the consecutive 8 d of the treatment period did not cause a notable further increase in the PAB level.

The concentration of PAB in the ruminal content remained high during the entire 5 days after the last treatment (Figure 1). For three of the cows (5979, 6043 and 6260), with ≤1% feed DM refusals, only a weak decline ($P < .034$) in PAB content was seen. A stronger reduction in ruminal PAB concentration was observed with cow 6322, the cow which had only an average total DMI of 17.7 and 19.1 kg per day in the treatment and posttreatment period, respectively. The reason for the feed refusal (grass silage) remains unknown, but it may have led to unfavorable conditions in the rumen for PAB maintenance or

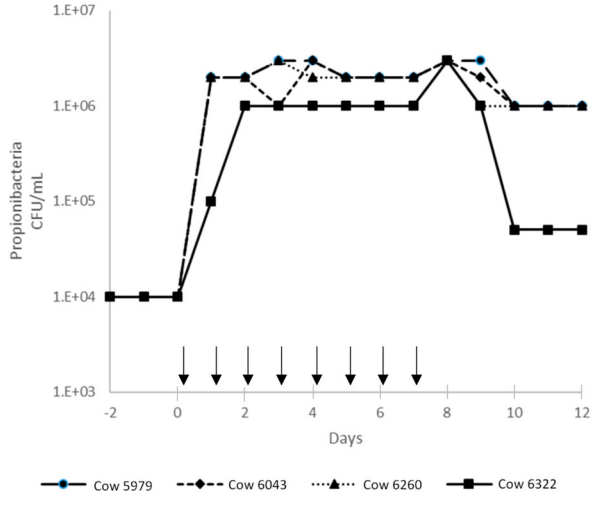

Figure 1. Kinetics of propionibacteria concentrations in the rumen of four cows. *Propionibacterium thoenii* strain T159 was administered through the rumen cannula on days 0–7 (indicated by arrows). Samples at days 1–8 were taken 24 h after administration of T159.

growth. The results of the present study demonstrated a comparatively long persistence of T159 in the rumen over at least 5 days post treatment. Previous work showed that T159 was detectable 9 h post treatment in the rumen of beef heifers (Vyas et al., 2016), but it was unclear if T159 can survive beyond 9 h and at high feed intake in the dairy cow rumen. The feeding level (DMI, % of body weight) in our study was about twice as high as in other reports administrating PAB to beef heifers (Vyas et al., 2014, 2016) and dairy cows (Jeyanathan et al., 2019). The rumen outflow rates of liquid and particles increase with increasing DM intake (Volden et al., 1998; Volden, 1999). Assuming a short rumen retention time due to the high feeding level, the low rate of disappearance from the rumen is a clear indication that the administrated PAB can grow in the rumen environment of dairy cows at high feeding level. At a high feeding level, the inoculant retention time is shorter and the supply of substrates for growth can be better, both factors favoring fast but not slow growing bacteria. Based on experiments comparable to the present study, rumen liquid and particulate outflow rates are expected to be between 15–20 and 8–10%/h, respectively (Volden et al., 1998; Volden, 1999). Our data show that the inoculated strain T159 was able to grow in the rumen faster than the outflow rate. With no growth the level of *Propionibacterium* would have been reduced to background levels within 3 d. To be stably established in the rumen the inoculant would have to grow with a mean doubling time of 3–5 h (unattached to particulates) or 7–9 h (attached to particulates). Albeit in laboratory media, a doubling time of 5 h has been reported for *P. thoenii* (Paik & Glatz, 1997). In addition to the ruminal survival of T159 in the post treatment period, the strain T159 could have stimulated other PAB strains in the rumen leading to the detected elevated PAB concentrations.

The present work showed that *Propionibacterium thoenii* strain T159 was able to persist for at least five days in the rumen of dairy cows with high feed intake. Recently, T159 was found to be promising for a substantial reduction of methane emissions per unit DM *in vitro* (Chen et al., 2020), but not in beef heifers with low feed intake (Vyas et al., 2016). Experiments with dairy cows with high feed intake still have to confirm the methane mitigating effect of the direct-fed microbial *Propionibacterium thoenii* strain T159.

Acknowledgements

We are grateful to the staff at the barn for their help during the animal trial.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was funded by Norwegian Research Council (Project 193613), Norwegian Agriculture Agency, Felleskjøpet Fôrutvikling AS, TINE SA, Animalia and Nortura SA.

ORCID

Helge Holo http://orcid.org/0000-0003-2563-0175
Angela Schwarm http://orcid.org/0000-0002-5750-2111

References

Bollet, C., Gevaudan, M. J., de Lamballerie, X., Zandotti, C. & de Micco, P. (1991). A simple method for the isolation of chromosomal DNA from gram positive or acid-fast bacteria. *Nucleic Acids Research*, 19, 1955.

Chen, J., Harstad, O. M., McAllister, T., Dörsch, P. & Holo, H. (2020). Propionic acid bacteria enhance ruminal feed degradation and reduce methane production in vitro. *Acta Agriculturae Scandinavica A*, doi:10.1080/09064702.2020.1737215

Davison, C. A. (1998). *The isolation, characterization and utilization of Propionibacterium as a direct fed microbial for beef cattle*. Master Thesis, Oklahoma State University.

Jeyanathan, J., Martin, C., Eugène, M., Ferlay, A., Popova, M. & Morgavi, D. P. (2019). Bacterial direct-fed microbials fail to reduce methane emissions in primiparous lactating dairy cows. *Journal of Animal Science and Biotechnology*, 10, 41.

Malik, A. C., Reinbold, G. W. & Vedamuthu, E. R. (1968). An evaluation of the taxonomy of *Propionibacterium*. *Canadian Journal of Microbiology*, 14, 1185–1191.

Paik, H.-D. & Glatz, B. A. (1997). Enhanced bacteriocin production by *Propionibacterium thoenii* in fed batch fermentation. *Journal of Food Protection*, 60, 1529–1533.

Stein, D. R., Allen, D. T., Perry, E. B., Bruner, J. C., Gates, K. W., Rehberger, T. G., Mertz, K., Jones, D. & Spicer, L. J. (2006). Effects of feeding propionibacteria to dairy cows on milk yield, milk components, and reproduction. *Journal of Dairy Science*, 89, 111–125.

Volden, H. (1999). Effect of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows. *Journal of Animal Science*, 77, 1905–1918.

Volden, H., Velle, W., Harstad, O. M., Aulie, A. & Sjaastad, Ø. V. (1998). Apparent ruminal degradation and rumen escape of lysine, methionine, and threonine administered intraruminally in mixtures to high-yielding cows. *Journal of Animal Science*, 76, 1232–1240.

Vyas, D., McGeough, E. J., Mohammed, R., McGinn, S. M., McAllister, T. A. & Beauchemin, K. A. (2014). Effects of *Propionibacterium* strains on ruminal fermentation, nutrient digestibility and methane emissions in beef cattle fed a corn grain finishing diet. *Animal*, 8, 1807–1815.

Vyas, D., Alazzeh, A. Y., McGinn, S. M., McAllister, T. A., Harstad, O. M., Holo, H. & Beauchemin, K. A. (2016). Enteric methane emissions in response to ruminal inoculation of *Propionibacterium* strains in beef cattle fed a mixed diet. *Animal Production Science*, 56, 1035–1040.

Weiss, W. P., Wyatt, D. J. & McKelvey, T. R. (2008). Effect of feeding propionibacteria on milk production by early lactation dairy cows. *Journal of Dairy Science*, 91, 646–652.