

Project number: 6341  
Funding source: Teagasc

Date: Apr 2018  
Project dates: January 2014 –2018

## Characterising the development of the rumen microbiome from birth to post weaning



Key external stakeholders:  
Nutritionists, dairy and beef farmers, Universities.

Practical implications for stakeholders:

- The rumen microbiome plays a key role in cattle performance and is linked to methane emissions and feed efficiency.
- Studies investigating rumen microbial manipulation as a means of improving performance report high resilience of the adult microbiome to change, but this may not be the case in the first days and weeks of life.
- We investigated the factors that influence the stepwise microbial colonization of the rumen from birth to post weaning.
- The data collected and presented in this project will contribute to the body of knowledge surrounding rumen microbial dynamics in cattle. This will further have major implications in the design of novel dietary strategies for rumen microbial manipulation in young calves.

### Main results:

Early life progression of microbial communities

- The rumen microbiome stabilises by the third week of life – for optimum effectiveness, dietary intervention should take place prior to this time.
- The patterns of microbial colonisation of the rumen are highly affected by farm environment – this must be considered in attempts to manipulate the microbiome.
- Major rumen microbial groups (e.g. *Prevotella*) were present within the first week of life, but became more dominant with increasing age.
- Gene expression in the rumen tissue of the host animal was also affected by age and diet, but not by farm, indicating the host animal is more resilient to external factors than the microbiome.
- Newborn calves did not have any microbial life in their rumen immediately after birth, indicating that colonisation occurs after birth from surrounding or maternal sources.

### Opportunity / Benefit:

- This is the first report to investigate external factors (including gender, age and environment) which may influence the rumen microbiota in early life.
- As part of an effort to design novel dietary/management strategies to permanently modify rumen fermentation, with a view to improve feed efficiency and/or reduce methane, these findings have significant implications on the critical timing of supplementation to gain permanent changes in the rumen microbiome.
- Early life remains the most promising window to alter rumen microbial development. However, our findings demonstrate novel determinants of microbial composition in early life, and these must be incorporated in future work.

### Collaborating Institutions:

University of Alberta, Canada.

**Teagasc project team:**

Dr Sinead Waters (Project Leader/PI)  
Dr David Kenny  
Eoin O'Hara

**External collaborators**

Prof. Le Luo Guan (University of Alberta)

---

**1. Project background:**

The rumen microbiome plays a central role in digestion and nutrient utilisation of feed by cattle and is thus critically important to beef and dairy production efficiency. Bacteria, fungi, methanogenic archaea, ciliate protozoa, and viruses are all found in the rumen, each comprising species with varied roles in plant biomass hydrolysis and subsequent end-product utilisation (Hobson and Stewart, 1997). Microbial diversity and activity in the rumen has been associated with variations in host feed efficiency (Jami et al., 2014, Carberry et al., 2012, Li and Guan, 2017), and methane formation (Kittelmann et al., 2014). Concerns as to the environmental impact of ruminant production have led a multitude of efforts to promote more energy efficient fermentation in the rumen by targeting the metabolism of certain groups of microorganisms, e.g. methanogens to reduce enteric CH<sub>4</sub> formation (Hristov, 2015). However, it has proven difficult to permanently modify the settled microbiota of the mature rumen, which generally reverts to the original composition following the cessation of treatment/supplementation (Weimer, 2015). There is some evidence that early interventions in the preweaned animal may have long-lasting effects on rumen microbial composition, but few studies have been conducted to-date (Yanez-Ruiz et al., 2010, Veneman et al., 2015, Krause et al., 2003). To properly identify the optimal timeframe for dietary interventions in early life, more comprehensive knowledge of microbial composition in the rumen in this period is required. This study addressed the gap in knowledge, and provided evidence of the role of the birthing process in the initial acquisition of gut microbiota by the ruminant.

---

**2. Questions addressed by the project:**

- How does the rumen microbiome establish and develop in early life?
- What factors influence this pattern of establishment?
- What is the optimum “window of opportunity” for manipulation of the rumen microbes?
- How do age and diet affect the rumen tissue transcriptome in early life?

---

**3. The experimental studies:**

- For the purposes of this study, 93 Aberdeen Angus (AA) crossbred heifers were purchased and artificially inseminated with semen from one easy-calving AA bull. A total of 67 became pregnant within the required time window, and randomly were assigned to one of 7 treatment groups.
- The 7 treatment groups included calves born via Caesarean section (c-section) and euthanised at birth, and calves born naturally (natural delivery), euthanised at intervals from birth to day 96 of life (i.e., day (d) 0 (natural delivery), d0 (c-section), d7, d14, d21, d56, d96 (post-weaning)) in order to identify any influence of the birthing process on the initial establishment of the rumen microbiota, and to characterise its development throughout the early life cycle, content and tissue samples of the rumen were collected at each time point.
- DNA was extracted from the rumen contents, and RNA from the rumen tissue collected at each time point. These were then sequenced to understand both host animal and microbial changes during early life.

---

**Main Results:**

- Clustering analysis of the microbial communities showed a clear separation of microbial composition in the rumen based on age, but a surprisingly large effect of farm environment on the rumen microbes.
- This suggests that calves raised on different farms have different microbial communities.
- Age had a major effect on microbial structure from day 7 to day 21, but there was no significant difference between 3- and 4-week old calves, indicating a stabilization of the microbial community in the first month. This suggests that the first three weeks might be an optimum time for manipulating the rumen.
- Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria were the dominant bacterial phyla throughout life, but their abundances changed dramatically with age. Day 7 animals had a large number of rare taxa, not associated with the normal rumen microbiota, but these had mostly disappeared by day 14.
- Known fibre degraders were detected within the first week, and the introduction of solid feed induced significant increases in important nutrient producing bacteria, thus contributing to rumen

development.

- These findings highlight the dynamic nature of the rumen microorganisms in early life, and show that the rumen bacteriome becomes relatively settled by week 3-4 weeks of life, but changes significantly in the post-weaned animal (d96).
- We examined the gene expression profiles (the transcriptome) in the rumen that occurred with increasing age and dietary change, finding that host gene expression response to diet and age was not as dramatic as the microbiome.
- We also observed large enrichment in immune-related processes in the host rumen tissue following birth, which may be a mechanism of tolerance to colonising microbes.
- When we examined the rumen of newborn calves, we could not find any microbial life present. This suggests that the rumen becomes colonised following birth, rather than during or before, by environmental microbes and those from other animals.

#### • Opportunity/Benefit:

- This is the first report to investigate external factors (gender, environment) which may influence the rumen microbiota in early life.
- As part of an effort to design novel dietary/management strategies to permanently modify rumen fermentation, with a view to improve feed efficiency and/or reduce methane, these findings have significant implications with regard to time of supplementation. For instance, given our data showed a settling of the microbiome by day 21, it is likely that manipulation must take place prior to this, for maximum effectiveness.
- Early life remains the most promising window of opportunity for rumen microbial manipulation, and our findings demonstrate novel determinants of microbial composition in early life (e.g. farm).

#### • Dissemination:

**PhD thesis:** Eoin O'Hara. Investigating early life microbial and host transcriptomic dynamics in the bovine gastrointestinal tract. December 2018. University of Alberta, Canada.

#### Conferences & Presentations

- 2018 – ANSC 575 guest lecture & tutorial: “*Amplicon sequencing to analyse microbial communities*”.
- 2018 – ASAS-CSAS Annual Meeting. *Poster & Oral*. Vancouver, Canada.
- 2018 – Joint Project Initiative Annual Meeting. *Oral*. Aberdeen, Scotland.
- 2018 – INRA-Rowett Symposium on Gut Microbiology. *Poster*. Aberdeen, Scotland.
- 2018 – Invited seminar: “*Climatic impact of livestock production*”. Universidad Autónoma Del Estado De Hidalgo, Tulancingo, Mexico
- 2018 – British Society of Animal Science Annual Meeting. *Poster*. Dublin, Ireland.
- 2018 – ANSC 318 guest lecture: “*Microbes and Animal Health*”.
- 2017 – AFNS 575 guest lecture & tutorial: “*Amplicon sequencing to study the microbiome*”.
- 2017 – Visiting scholar seminar: “*The rumen microbiome during early life*”. Hokkaido University, Sapporo, Japan.
- 2016 – Joint Project Initiative Annual Meeting. *Oral*. Dublin, Ireland.
- 2016 – ASAS/ADSA/CSAS Joint Annual Meeting. *Poster*. Salt Lake City, USA.
- 2016 – INRA-Rowett Symposium on Gut Microbiology. *Poster*. Clermont-Ferrand, France.
- Regular participant in journal club at Teagasc Grange, Ireland. 2014-2017.
- Regular presentations to students and researchers at the University of Alberta, Canada. 2014-2018.

#### Awards & Recognition

- 2018 – CSAS Graduate Student Travel Fellowship (Canadian Society of Animal Science)
- 2018 – CSAS PhD Student Oral Presentation – 2<sup>nd</sup> prize (ASAS-CSAS Annual Meeting)
- 2018 – Graduate Students Association Travel Award (University of Alberta)
- 2018 – FEMS ECR Meeting Attendance Grant (INRA-Rowett, France/Scotland).
- 2017 – Scholarship for Short-term Study Abroad (Hokkaido University, Japan).
- 2016 – AFNS Graduate Student Travel Award (University of Alberta, Canada).
- 2016 – CSAS PhD Student Poster Presentation – 1<sup>st</sup> prize (ASAS-CSAS-ADSA Annual Meeting).

#### Publications

#### Peer reviewed:

- **O'Hara E**, Kelly AK, McCabe MS, Kenny DA, Guan LL, Waters SM. 2018. Effect of a butyrate-

fortified milk replacer on gastrointestinal microbiota and products of fermentation in artificially reared dairy calves during the peri-weaning period. *Nat Sci Rep.* 8:14901

- Surlis C, McNamara K, **O'Hara E**, Waters S, Beltman M, Cassidy J, Kenny D. 2017. Birth delivery method affects expression of immune genes in lung and jejunum tissue of neonatal beef calves. *BMC Vet Res.* 13(1):391.
- McCabe MS, Cormican P, Keogh K, O'Connor A, **O'Hara E**, Palladino RA, Kenny DA, Waters SM. 2015. Illumina MiSeq phylogenetic amplicon sequencing shows a large reduction of an uncharacterised Succinivibrionaceae and an increase of the *Methanibrevibacter gottschalkii* clade in feed restricted cattle. *PLoS One.* 10(7): e0133234

#### In preparation:

- **O'Hara E**<sup>\*</sup>, Zhou M<sup>\*</sup>, Tang S, Chen Y, Walpole ME, Górká P, Woodbury M, Penner GP, Guan LL. 2018. Assessing dietary effects on the rumen microbiome in bull cattle: different sequencing methods tell different stories. *Under review at Frontiers in Microbiology.* <sup>\*</sup>Co-first authorship
- **O'Hara E**, Kenny, DA, Byrne C, McCabe MS, McGovern E, Guan, LL, Waters, SM. Diet, age, and farm environment influence rumen microbial establishment patterns during early life.
- **O'Hara E**, Kenny DA, Beltman M, Chen Y, McGovern E, Guan LL, Waters SM: Dynamics of the rumen wall transcriptome during early life.
- Lyons T, Hanne J, Brady J, **O'Hara E**, Waters SM, Doyle E, Meade K. Influence of gut microbes on the host immune system in early life.

#### Conference proceedings:

- **O'Hara E**, Kelly AK, McCabe MS, Kenny, DA, Guan LL, Waters SM. 2018. Effect of a butyrate-fortified milk replacer on gastrointestinal microbiota and products of fermentation in artificially reared dairy calves at weaning. Proc. ASAS-CSAS Annual Meeting, Vancouver, Canada. Online
- **O'Hara E**, Kenny, DA, McGovern E, Waters SM. 2018. Temporal development of the rumen microbiome in beef calves from birth to post weaning. Proc. ASAS-CSAS Annual Meeting, Vancouver, Canada. Online
- **O'Hara E**, Kenny, DA, McGovern E, Waters SM. 2018. Temporal development of the rumen microbiome in beef calves from birth to post weaning. Proc. BSAS Annual Meeting, Dublin, Ireland, p. 65.
- **O'Hara E**, Kenny, DA, McGovern E, Waters SM. 2018. Temporal development of the rumen microbiome in beef calves from birth to post weaning. Proc. INRA-Rowett Symposium on Gut Microbiology. Aberdeen, Scotland. Online.
- Lyons T, Hanne J, Brady J, **O'Hara E**, Waters S, Doyle E, Meade K. Influence of gut microbes on the host immune system in early life. Proc. INRA-Rowett Symposium on Gut Microbiology. Aberdeen, Scotland. Online.
- **O'Hara E**, Zhou M, Waters SM, Walpole ME, Gorka P, Woodbury M, Penner GB, Guan LL. 2016. Taxonomic assessment of the rumen microbiome of bulls under backgrounding and finishing diets. Proc. ADSA/ASAS/CSAS Joint Annual Meeting, p. 97.
- **O'Hara E**, Kelly AK, McCabe MS, Cormican P, Kenny, DA, Guan LL, Waters SM. 2018. Investigation of the effect of dietary butyrate on rumen and hindgut microbial communities and fermentation products in pre-weaned calves. Proc. INRA-Rowett Gut Microbiology Symposium, Clermont-Ferrand, France.

- **Compiled by:** Dr. Sinéad Waters, Eóin O'Hara