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'RumenStability' Understanding the development and control of stability in the rumen microbiome as a basis for new strategies to reduce methanogenesis

Key external stakeholders:
Livestock industry, Universities, Veterinarians, Department of Agriculture, Food and the Marine, Animal Nutrition companies.

Practical implications for stakeholders:

- The quantity of feed consumed can evoke large effects on the composition of methanogenically active microbial species in the rumen of cattle. These data potentially have major implications for targeted CH_4 mitigation approaches such as anti-methanogen vaccines and/or tailored dietary management.
- Host animal feed efficiency (FE) (measured as residual feed intake or RFI) phenotype andn diet fed affects the rumen microflora of cattle possibly enhancing the nutrient utilisation from feed.
- The effect of host FE on the rumen microbial population also appears to be dependent host animal genetics suggesting that breeding strategies could alter the rumen microbiome to enhance feed efficiency.
- The abundance of certain bacterial genera such as *Fibrobacter* in the rumen exhibited relationships with RFI phenotype possibly due to their role in ruminal degradation of complex plant polysaccharides or increased capability to harvest nutrients from ingested feed. Supplementation of feed with *Fibrobacter* could enhance nutrient utilization from feed.
- Abundance *Methanobrevibacter* Y315 may be an indicator of host FE in cattle fed a high concentrate diet as it appears to thrive in a rumen ecosystem that is sustained by a feed efficient phenotype irrespective of breed or age, and can thus contribute to a breeding strategies to enhance feed efficiency and lower methane emissions.

Main results:

- The quantity of feed consumed can evoke large effects on the composition and diversity of transcriptionally active methanogens (such as *Methanobrevibacter gottschalkii*) in the rumen of cattle.
- Microbial sequencing analysis of the rumen digesta has provided evidence that the abundance of certain bacterial genera such as *Fibrobacter,* exhibit relationships with RFI phenotype. It is hypothesized that this is due to their role in ruminal degradation of complex plant polysaccharides or increased capability to harvest nutrients from ingested feed.
- Ruminant FE phenotype influences the rumen microbial ecosystem, though this effect appears to be dependent on diet type and host animal genetics when steers are growing.
- *Methanobrevibacter* Y315 shows a continuous negative relationship with RFI in the rumen fluid of cattle offered a concentrate diet irrespective of breed or age.

Opportunity / Benefit:

- These data could potentially have major implications for targeted CH_4 mitigation approaches such as anti-methanogen vaccines and/or tailored dietary management strategies.
- Supplementation of feed with *Fibrobacter* could enhance nutrient utilization from feed.
- Abundance of this *Methanobrevibacter* Y315 may be an indicator of host FE in animals fed a high concentrate diet as it appears to thrive in a rumen ecosystem that is sustained by a feed efficient phenotype irrespective of breed or age. This information can contribute to a breeding strategy to enhance feed efficiency and lower methane emissions.

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Collaborating Institutions:
UCD, INRA

Teagasc project team: Dr Sinead Waters (Project Leader/PI)
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1. Project background:

The rumen microbiome consists of many microbial species working together to convert low value lignocellulosic plant material into volatile fatty acids, which are the main energy source of the host ruminant in a process called rumen fermentation. However, this process is accompanied by the production of anthropogenic methane which has major environmental consequences. Rumen fermentation is a critical process in light of the rising global human population and intensifying demand for animal protein. There is a requirement therefore to produce beef cattle with enhanced nutrient utilization capacity in order to more efficiently convert plant material to high quality edible muscle to enhance beef production and profitability. This drive to enhance livestock productivity is also expected to reduce anthropogenic methane production therefore increasing the sustainability of the livestock sector. Elucidation of the relationship between the host ruminant and rumen microbiota in response to diet may help to determine if trait improvement can be achieved via microbial manipulation or genetic selection based on rumen microbiome composition. As well as innate variation between animals fed to appetite, feed efficiencies may also be enhanced in cattle through nutritional management practices such as those associated with compensatory growth. A period of moderate feed restriction followed by *ad libitum* access to feed is widely applied in cattle management to exploit the animal's compensatory growth potential and reduce feed costs. Compensatory growth (CG) could be conceptualised as an extension or extreme feed efficiency. However, there is a shortage of information surrounding the biological control governing the expression of the trait and its effects on the rumen microflora and in particular the methanogen or methane producing microbial community.

2. Questions addressed by the project:

- Which methanogens contribute to methanogenesis in the bovine rumen during periods of dietary restriction and *ad libitum* feeding?
- Do rumen microbes influence host feed efficiency phenotype and does this change when different diets are fed and for different cattle breeds?
- Are specific microbial species associated with host feed efficiency and is that relationship consistent when different diets are fed and for different breeds of cattle?

3. The experimental studies:

1. Compensatory Growth model

Holstein Friesian bulls (n=60) were divided into two groups and subjected to one of two dietary regimes (n=30). One group was subjected to an *ad libitum* diet and the other group was subjected to a restricted diet for a total of 125 days. After this period, 15 animals from each group were slaughtered. The remaining 15 animals from both groups were then offered an *ad libitum* diet for a further 55 days and then slaughtered after this period. All animals were offered the same diet consisting of 70:30 concentrate:forage, with restricted animals receiving a restricted ration compared to *ad libitum* fed animals. Rumen digesta samples were collected immediately after slaughter.

2. Residual Feed intake (RFI) model

Residual feed intake was calculated for each animal as the difference between actual dry matter intake (DMI) and expected DMI for a combination of four cohorts of Simmental bulls (n=87) over four years. All animals were managed similarly from birth and offered *ad libitum* concentrate and 3kg of grass silage daily during the finishing period. Liquid and solid rumen digesta were collected immediately after slaughter.

3. RFI x Diet x Genotype model

Residual feed intake was calculated for each animal as the difference between actual dry matter intake (DMI) and expected DMI for 167 cattle comprised of 90 CH and 77 HF. Individual dry matter intake (DMI)

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and growth were measured over three 70 d feeding phases which included; high concentrate phase, grass silage, zero grazed grass and a second high concentrate phase. During each experimental phase a rumen fluid sample was collected for each animal via stomach intubation for metabolite profiling and microbial analysis.

Measurements:
The volatile fatty acids (VFA) concentration of rumen fluid for all experiments was measured using gas chromatography. Amplicon sequencing targeting bacterial and archaeal microbial populations was conducted for all three experiments utilizing the Illumina MiSeq. Bioinformatic analyses was conducted to establish the effects of treatment on the diversity, abundance and activity of bacterial and archael microflora in the rumen.

4. Main results:

- When compared to their unrestricted contemporaries, in feed-restricted animals, the methanogenic activity, of *Methanobrevibacter gottschalkii* clade increased while the methanogenic activity of the *Methanobrevibacter ruminantium* clade and members of the *Methanomassiliicoccaceae* family decreased. This highlighted that the quantity of feed consumed can evoke large effects on the composition and diversity of transcriptionally active methanogens in the rumen of cattle.
- 16S rRNA of the rumen digesta has provided evidence that the abundance of certain bacterial genera such as *Fibrobacter* exhibit relationships with RFI phenotype. It is hypothesized that this is due to their role in ruminal degradation of complex plant polysaccharides or increased capability to harvest nutrients from ingested feed
- *Methanobrevibacter* Y315 shows a continuous negative relationship with RFI in the rumen liquor of cattle offered a concentrate diet irrespective of breed or age.

5. Opportunity/Benefit:

- Supplementation of feed with *Fibrobacter* could enhance nutrient utilization from feed.
- Abundance of this *Methanobrevibacter* Y315 may be an indicator of host FE in animals fed a high concentrate diet as it appears to thrive in the ecosystem that is sustained by a LRFI phenotype irrespective of host genotype.
- The data potentially has major implications for targeted CH_4 mitigation approaches such as anti-methanogen vaccines and/or tailored dietary management strategies.

6. Dissemination:

PhD thesis:
E. McGovern. An investigation into the relationship between bovine feed efficiency and rumen archaeal and bacterial populations. University College Dublin. Awarded August 2018.

Main publications:

- McGovern E, Kenny DA, McCabe MS, Fitzsimons C, McGee M, Kelly AK, Waters SM. 16S rRNA Sequencing Reveals Relationship Between Potent Cellulolytic Genera and Feed Efficiency in the Rumen of Bulls. Front Microbiol. 2018 Aug 10;9:1842.
- McGovern E, Waters SM , Blackshields G, McCabe MS. 2018. Evaluating established methods for rumen 16S rRNA amplicon sequencing with mock microbial populations. Frontiers in microbiology. 9:1365.
- McGovern E, McCabe MS, Cormican P, Keogh K, Popova M, Kelly AK, Kenny DA, Waters SM. Plane of nutrition affects the phylogenetic diversity and relative abundance of transcriptionally active methanogens in the bovine rumen. Scientific reports 2017, 7(1): 13047.

International invited presentations:

- Waters SM. *2018. Bovine feed efficiency and the rumen microbiome. 2nd International Symposium on Young Ruminant Rearing (ISYR) Nutritional Regulation & Environmental Interaction, (Beijing China). October 22nd.*
- *Waters SM. 2017. Role of the rumen Microbiome in feed efficiency and methane emissions in the bovine. 2nd IS-FOOD International* Workshop, Public University of Navarra, Pamplona, Spain. 8th June, 2017).

Conference Proceedings:

- McGovern E, *et al* (2018) Characterisation of the rumen archaeal and bacterial populations in bulls offered a high concentrate diet phenotypically divergent for residual feed intake. BSAS, Dublin, Ireland,

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2018
- McGovern E, *et al* (2017) 16S rRNA amplicon sequencing of mock microbial populations to investigate DNA extraction methodology, primer selection and PCR cycles. ISAG, Dublin, Ireland, 2017
- McGovern*, et al* (2017). Feed restriction provides a niche environment for metabolic activity of the *Methanobrevibacter gottschalkii* clade in the bovine rumen. BSAS, Chester, UK
- McGovern E *et al*. (2016) Understanding the development and control of stability in the rumen microbiome as a basis for new strategies to reduce methanogenesis. JPI Workshop, Clermont Ferrand, France.
- McGovern E *et al*. (2016) Methanogensis in the bovine rumen under conditions of dietary restriction and subsequent compensatory growth. 20th INRA-Rowett, Clermont-Ferrand, France.
- McGovern E *et al*. (2015) Understanding the development of rumen methanogen populations during key life cycle events in ruminants – the birthing process, weaning and dietary transitions. JPI Workshop, Malaga, Spain.

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