# Temporal establishment of the colon microbiota in Angus calves from birth to post-weaning

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## Introduction

- The **hindgut** microbiome remains **largely unexplored** in ruminants and plays a key role in health and productivity.
- A range of commensal microbes within the gastrointestinal tract (GIT) function in processes including **immune development, nutrient supply, prevention of pathogenic diseases** and **overall health** (Myer et al., 2015, Arshad et al., 2021, Xu et al., 2021, Zhang et al., 2021).
- **Diarrhoea** in neonatal calves has a severe **economic impact** on the beef and dairy industries. Veterinary interventions together with **calf morbidity, mortality** and latent effects on **health and performance** are notable costs (Carter et al., 2021).
- Initial post-natal **host-microbe interactions** are fundamental to the programming and development of the **adaptive immune** system (Malmuthuge et al., 2015, Lyons et al., 2020,).

## Objective

The aim of this research was to characterise the ontogeny of microbial establishment within the colon digesta from birth until the early post-weaning period.

## Materials and Methods

### Animal Model

Commercially purchased Aberdeen Angus heifers housed in Teagasc Athenry (Farm 1)

7-day PRID Oestrous synchronisation

AI served from a single Aberdeen Angus bull sire

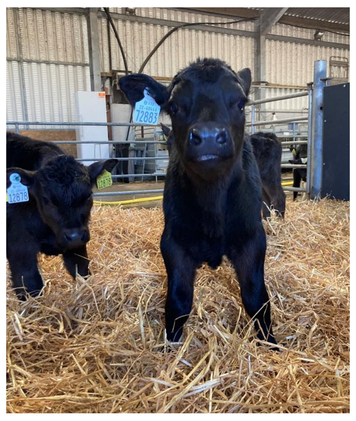
Heifers in duplicate were transported to Longtown research (Farm 2) during the 3rd trimester

Blocked by foetal sex

Day 0 (n=7) | Day 7 (n=7) | Day 14 (n=5) | Day 21 (n=7) | Day 28 (n=5) | Day 96 (n=7)

Each calf was delivered transvaginally. Calves not allocated to the Day 0 cohort were allowed to suckle their dam for 48 hours. Calves in group D7-D96 were fed 5L milk replacer and allocated increasing amounts of calf starter. D96 calves were weaned on D56 and received hay and calf starter on an *ad libitum* basis. As described in O'Hara et al., 2018.

### Sample collection



Calves were euthanised using a intravenous injection of pentobarbital sodium using the recommended dosage. Samples were collected in a clean room in sterile vessels using sterilised instruments. The colon was tied distal to the cecal junction and digesta pushed through. Colon digesta samples collected were snap frozen in liquid nitrogen and stored in a -80°C freezer.

### Microbial Analysis

Microbial DNA was extracted using repeated bead method (as described in Yu and Morrison, 2004)

V4 region of 16S rRNA gene amplified and sequenced on Illumina MiSeq

Amplicon Sequencing. Exactly.

Amplicon sequence data analysed in *R* package vegan and Maaslin 2

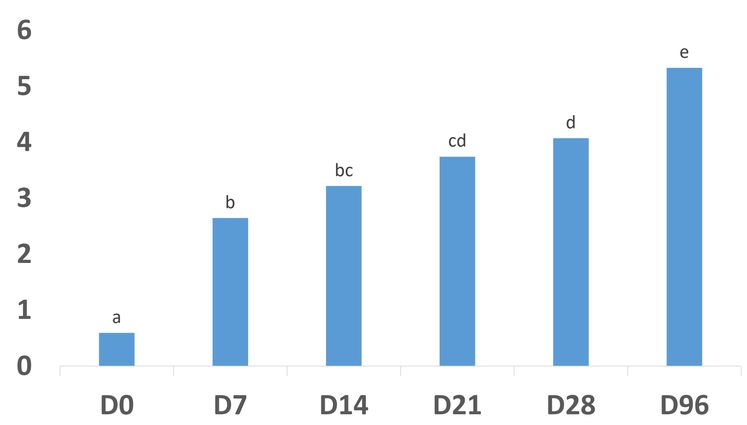
## Results

### Beta Diversity Analysis

| Factor | DF[1] | SumOfSqs | $R^2$ | *P*-Value |
|---|---|---|---|---|
| Age | 4 | 3.4877 | 0.30137 | 0.001 |
| Farm | 1 | 0.8101 | 0.7000 | 0.001 |
| Residual | 25 | 7.2751 | 0.62863 | |
| Total | 30 | 11.5729 | 1.00000 | |

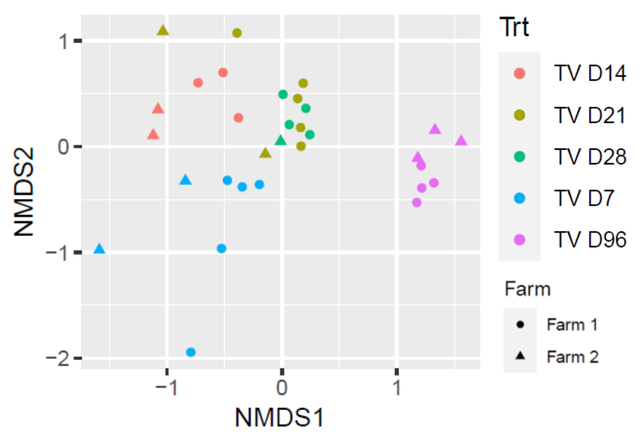
[1] DF – Degrees of freedom
[2] Pseudo-F value, obtained by permutation

### Shannon LSMean




[a,b,c,d,e] least square means with age group affect and age with different superscripts differ

### Bray NMDS




## Conclusion

Stabilisation of the colon microbial composition occurred from D14 to D21 (D14 vs D21; P= 0.22) with weaning also affecting the microbial composition (D28 vs D96; P<0.05).

The findings from this study are amongst the first to describe the ontogeny of colonisation of the colon microbiota, from birth until weaning in beef calves, and provides fundamental information on the potential to promote the establishment of beneficial microbes in the lower GIT of ruminants.

## Acknowledgments

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