**Application**

The colon plays a pivotal role in early life nutrition, performance and health of calves, particularly during the pseudo-monogastric phase. Optimising the early establishment of a resilient microbiome helps limit the infiltration of pathogens and reduces disease incidence, while enabling better growth and performance in neonatal calves.

**Introduction**

The colonisation of the gastrointestinal microbiome begins shortly after parturition and is central to modulating host defence mechanisms and contributes to the regulation of inflammatory response. Early microbial colonisation of the lower GIT is influenced by numerous factors including maternal microbiota, age, diet, weaning and environmental factors (Amin and Seifert, 2021). This early microbial colonisation supports the production of various pre-immune B cells and the expression of tight junction proteins, which are protective mucosal barriers (Malmuthuge et al., 2013). The neonatal lower GIT microbial community is a diverse and volatile environment. Therefore, the aim of this study was to characterise the temporal establishment and dynamics of the bacterial community of the colon digesta of Aberdeen Angus calves using DNA amplicon sequencing of the 16S rRNA gene from birth to post-weaning.

**Materials and Methods**

Animal management protocols were previously described by Surlis et al. (2017) and O'Hara et al. (2020). Heifers were subject to AI from a single Aberdeen Angus Bull sire, 42 heifers utilised for the experiment were housed in Teagasc Mellows Campus, Athenry, Co. Galway, Ireland (Farm 1; F1). During the third trimester, a subset of heifers (n = 18) were transported to the Department of Agriculture, Food and Marine (DAFM) Longtown Research Facility in Clane, Co. Kildare, Ireland (Farm 2; F2) for calving. Both facilities adhered to the same housing and feeding practices. All calves were delivered transvaginally. The calves assigned to the D0 cohort had no contact with the cow or their external environment following birth. Calves not in the D0 cohort, nursed from their dam for 48 hours before being housed in individual pens. Calves were euthanised via lethal intravenous injections of pentobarbital sodium, according to their allocated groupings. Death was determined by the absence of cardiac activity and the lack of a corneal reflex. The colon digesta samples were collected from calves at day 0 (D0, n=7), D7 (n=7), D14 (n=5), D21 (n=7), D28 (n=5), and D96 (n=7) of life. Samples were snap frozen in liquid nitrogen and stored at -80oC. The DNA was extracted from approximately 250 mg of ground frozen sample, using a modified version of repeated bead beating and column purification method (Yu and Morrison, 2004) and as previously described (Smith et al., 2022). Sequencing was carried out on the Illumina MiSeq platform.



Figure 1. Bacterial genus abundance of the colon digesta at different time points during early life. Each bar represents an individual animal host and the x-axis shows the host ID. The y-axis depicts the proportion of the sum of the 20 most abundant bacterial genera.

**Results**

Alpha and beta diversity were assessed using GLM (SAS 9.4) and PERMANOVA, with age and farm as fixed effects. Bacterial α-diversity increased significantly with age, showing marked differences between D7 and later stages, including D21, D28, and D96 (each p < 0.0001), indicating substantial shifts in microbial diversity as the calves mature. Microbial colonization began to stabilize between D14 and D21 (p = 0.22), suggesting an early-stage equilibrium in microbial composition. Weaning further altered the microbiota, with significant diversity differences between D28 and D96 (p < 0.001). PERMANOVA analysis shows that both age (P < 0.001) and farm (P < 0.001) affect bacterial community composition in calves, with age explaining the largest proportion of variance.

**Conclusions**

The neonatal GIT microbiome is a highly complex environment that plays a pivotal role in immune development, nutrient absorption and disease resistance. This study demonstrates the considerable changes the colon digesta microbiota undergoes in response to various factors such as the environment and dietary changes particularly as the calf transitions from a milk-based to solid-feed diet.

**References**

MALMUTHUGE, N., LI, M., GOONEWARDENE, L. A., OBA, M. & GUAN, L. L. 2013. Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. *Journal of Dairy Science,* 96**,** 3189-3200.

O'HARA, E., KENNY, D. A., MCGOVERN, E., BYRNE, C. J., MCCABE, M. S., GUAN, L. L., et al. 2020. Investigating temporal microbial dynamics in the rumen of beef calves raised on two farms during early life. *FEMS Microbiol Ecol,* 96.

SMITH, P. E., KELLY, A. K., KENNY, D. A. & WATERS, S. M. 2022. Differences in the Composition of the Rumen Microbiota of Finishing Beef Cattle Divergently Ranked for Residual Methane Emissions. *Frontiers in Microbiology,* 13.

SURLIS, C., MCNAMARA, K., O’HARA, E., WATERS, S., BELTMAN, M., CASSIDY, J., et al. 2017. Birth delivery method affects expression of immune genes in lung and jejunum tissue of neonatal beef calves. *BMC Veterinary Research,* 13**,** 391.

YU, Z. & MORRISON, M. 2004. Comparisons of Different Hypervariable Regions of rrs Genes for Use in Fingerprinting of Microbial Communities by PCR-Denaturing Gradient Gel Electrophoresis. *Applied and Environmental Microbiology,* 70**,** 4800-4806.