**The faecal microbiota during the pre-weaning period and its relationship with diarrhoea, health and performance in dairy heifer calves**

**Application:** Depicting dysbiosis of the hindgut microbiota provides a better understanding of calves with diarrhoea and should facilitate the development of prevention strategies and improvement of animal health and welfare.

**Introduction:** Diarrhoea is a complex disease that results in significant economic burdens to the dairy industry. The relationship between the developing hindgut microbiome and the onset of diarrhoea requires deeper understanding. Many factors influence the development and colonisation of the hindgut microbiome during the pre-weaning period. At this time, the microbiome is unstable and sensitive to disturbances, which can result in dysbiosis, in that a loss in commensal microbes occurs in conjunction with the proliferation of pathogenic species (Chase and Kaushik, 2019). Dysbiosis has been previously linked to diarrhoea and it has been suggested that diarrhoea is the cause of this imbalance (Ma et al., 2020). To better understand the relationships between bacteria within the faecal microbiome pre-, during, and post-disease, a correlation analysis of bacterial genera found to be significant due to health status was performed to better characterize the dysbiosis of the hindgut microbiota and determine if it is the cause or result of diarrhoea in neonatal calves.

**Materials and methods:** Fifty-one spring born Holstein (n=29, birth weight (BW) 34.7 (SE 0.69) kg) and Jersey (n=22, BW 25.9 (SE 0.81) kg) heifer calves were observed from birth (day (d) 0) until weaning (d83 (SE 1.04)). Calves were fed 8.5% BW in colostrum, from either, the calf’s dam (n=28) or a mixed source of colostrum (≤2 cows, ≤1d; n=23) within 2 hours of birth. Blood was collected via jugular venepuncture at d7 and analysed for serum immunoglobulin G (sIgG) using single radial immunodiffusion. Calf clinical assessments were performed at d7, d21 or disease manifestation, and d83 using a Modified Wisconsin-Madison scoring method. Faecal samples were collected during clinical assessment. Diarrhoea incident was determined using faecal scores and health status (HS) was defined as calves having diarrhoea (n=27) or healthy (n=24). Microbial DNA was extracted from 150 faecal samples using a Qiagen DNeasy® PowerSoil® Pro Kit and extracted DNA was sent to Macrogen for 16S amplicon sequencing. Amplicon sequence data were processed in *R* using *DADA2* and taxonomy assigned using the SILVA database. Data were further analysed using the *R* packages *Phyloseq* and *MaAsLin2*, and the Benjamini-Hochberg procedure (q) was used to correct for false discovery rate. Amplicon Sequence Variants (ASVs) found associated with HS were checked for normality and homogeneity of variance by histograms, qqplots, and formal statistical tests as part of the UNIVARIATE procedure in SAS 9.4. A Spearman rank-order correlation for non-parametric data was preformed to determine correlations between ASVs and calf performance data including faecal scores. Correlations with a P-value less than 0.05 were considered significant.

**Results:** The mean day post-birth for diarrhoea was d22 (SE 0.70); 53% of calves had a diarrhoea incident. There was no difference (P>0.05) in sIgG between healthy and diarrheic calves (Healthy: 43.4 (SE 1.82) g/L; Diarrheic: 37.6 (SE 2.52) g/L). Based on PERMANOVA analysis, calf breed (P=0.08), colostrum source (P=0.31), and passive immune status (P=0.21) had no effect on composition of the faecal microbiota. At disease manifestation, diarrheic calves had reduced bacterial diversity compared to healthy calves, and 24 ASVs were significantly associated (q< 0.05) with HS. *Bifidobacterium* (6.4%) was the dominant genera in healthy calves, while *Alloprevotella* (13.7%)was the dominant genera in diarrheic calves at disease manifestation. *Alloprevotella* had strong negative correlations (P≤0.0001) with *Faecalicoccus, Akkermansia,* and *Intestinibacter*, and a moderate negative correlation (P≤0.01) with *Bifidobacterium, Prevotella\_7, Flavonifractor,* and *Dialister.* *Bifidobacterium* had a strong positive correlation (P≤0.0001) with *Prevotella\_7* and moderate positive correlations (P≤0.01) with *Collinsella, Faecalicoccus, Flavonifractor, Dialister,* and *Intestinibacter*. A weak negative correlation was observed between sIgG and *Alloprevotella* and moderate positive correlations with *Prevotella\_7* and *Faecalicoccus*. Faecal Score displayed moderate negative correlations (P≤0.01) with *Bifidobacterium, Prevotella\_7, Faecalicoccus, Dialister,* and *Intestinibacter.* A strong positive correlation between faecal score and *Alloprevotella* was observed. No significant correlations were found between ASVs and average daily gain.

**Conclusion:** The reduction in microbial diversity observed in diarrheic calves enabled the proliferation of other bacterial genera. *Alloprevotella*, has not previously been associated with diarrhoea in calves, and is considered a part of the normal intestinal flora. The proliferation of *Alloprevotella* appears to be negatively associated with other genera that are known to be beneficial to gut health (e.g. *Bifidobacterium, Faecalicoccus*)*.* The correlations between ASVs significant at disease manifestation show the relationship between microbes in the hindgut and the influence they have on each other as their presence changes within the hindgut.

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**References:**

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