**Application**

This project was funded by the BSAS Steve Bishop Net Zero Award. This pilot study investigates the parasitic gastrointestinal nematode burden and the faecal microbiota of weaned lambs grazing two sward types. Exploring the interaction between parasite load, diet and the gut microbiome may help in our understanding of host-susceptibility to infection.

**Introduction**

Parasitic gastrointestinal nematodes (GIN) can negatively impact animal health and production and can be an important constraint to efficient sheep production. GINs share their environment with the gastrointestinal microbiome. GINs induce pathological changes to the gut mucosa and their presence is likely to impact upon the microbial community. Diet is also a distinct driver of diversity and abundance of intestinal microbiome.

Previously, sheep experimentally infected with *Haemonchus contortus* have been shown to exhibit a variation in gut bacterial composition associated with high or low levels of infection (Mamun et al., 2020). However, this association has not been explored in naturally infected animals who will inevitably harbour mixed GIN infection. Therefore, this preliminary study was designed to investigate the gut microbiome of weaned lambs naturally infected with gastro-intestinal parasites grazing two different sward types.

**Materials and Methods**

Naturally voided faecal samples were collected from five Suffolk mule X Charollais cross lambs grazing four plots, established with two pasture types (perennial rye grass, and a mixed sward of 2 grasses, 2 legumes and 3 forbs), at three post-weaning time points between July and September (d0, and d35 and d57). Sixty faecal samples were collected from the ground into sterilised labelled plastic pots and transferred back to the lab for analysis. From each faecal pat, three sub samples (0.5g) were transferred into sterilised 3ml Eppendorf tubes containing 2ml RNAlater. These were left at room temperature for 24h then stored at -20°C until further analysis. The remaining sample was used for faecal egg counts (FEC).

Faecal egg counts were determined using the FECPAK S5 method (Techion Ltd, UK), the presence of coccidia, tapeworm and strongyloides were recorded. DNA was extracted using the prepGEM Bacteria kit (MicroGEM, Charlottesville, VA, USA). Two-step PCR was performed targeting the V4 region of 16S rRNA gene. During the second PCR, sample-indexing was performed using the Nextera ® XT Index Kit (Illumina, Inc., San Diego, CA, USA). Final libraries were normalised to 4 nM and sequenced on an Illumina MiSeq platform (paired 300 bp reads).

Sequencing reads were processed using QIIME2 2024.5.0 {Bolyen, 2019 #65008} to obtain a list of amplicon sequencing variants (ASVs) for each sample. The ASV taxonomy was classified by training a naïve-Bayes classifier on the SILVA reference database in release version v.138 {Quast, 2012 #65016} for 16S rRNA gene sequences, and ASVs assigned to chloroplasts or mitochondria were removed. Chao1 and Shannon Diversity indices from rarified samples were used to estimate microbial per-capita species richness and diversity.

For the egg count data, generalised linear mixed effect models were fitted using the ‘glmmTMB’ function for R (R core team 2015). The number of nematode eggs detected was the response variable, and explanatory variables were month of sampling and sward type with individual lamb as random effect. September and mixed sward were set as the reference categories within the respective fixed effects. Fixed effects were considered significant when p<0.05, Tukey post-hoc test was used for multi-group comparisons.

**Results**

Lambs had significantly lower parasite burden in July than in August (p<0.05) and September (p<0.05). There was no evident difference in parasite burden between August and September (p=0.6). The glmm indicated lambs grazing rye grass had significantly lower nematode burden than those grazing the mixed sward (p<0.05).

Preliminary results of Chao1 species richness estimates, and Shannon Diversity index, suggest that gut microbial alpha diversity marginally increased from July to August (p < 0.05) and from July to September in weaned lambs. We found no evidence that alpha diversity measures varied across pasture type or with parasite egg counts.

**Conclusions**

Faecal egg counts varied greatly between individual animals, and sampling occasions. Gut microbial diversity varied over time. Further analysis is necessary to explore possible relationships between parasite burden and the relative abundance of gastrointestinal bacteria.

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