

Bacterial populations in the sheep gastrointestinal tract—variation with resistance to helminths

E. A. Paz^{A,D}, E. G. Chua^B, J. C. Greeff^{A,C}, S. U. Hassan^{A,B}, D. G. Palmer^C, S. Liu^A, B. Lamichhane^B, C. Y. Tay^B and G.B. Martin^A

^AUWA Institute of Agriculture, University of Western Australia, Crawley, WA 6009, Australia.

^BMarshall Centre for Infectious Disease Research and Training, School of Biomedical Sciences, University of Western Australia, Crawley, WA 6009, Australia.

^CDepartment of Primary Industries and Regional Development, Western Australia, South Perth, WA 6151, Australia.

^DEmail: erwin.pazmunoz@uwa.edu.au

Nematode parasitism is a major problem that affects the sheep industry worldwide. It has been well recognized that infection with gastrointestinal nematodes (helminths) changes the intestinal microenvironment (Mamun *et al.* 2020) with likely consequences for gastrointestinal microbial populations. We tested this hypothesis by investigating the diversity of microbiota along the gastrointestinal tract of Merino sheep that had been bred for 25 years for resistance to helminths (Greeff and Karlsson 2020). Our aim was to determine the sections tract in which the microbial populations differed between worm-resistant and worm-susceptible sheep.

After weaning, 10 highly resistant (R) and 10 highly susceptible (S) sheep were selected on basis of their breeding values for faecal worm egg count (WEC). The sheep were slaughtered at hogget ages and luminal contents were collected from the rumen, abomasum, duodenum, jejunum, ileum, colon, caecum and rectum. DNA was extracted and sequenced for the V3-V4 hypervariable region of the 16 rRNA gene on Illumina MiSeq using 300 bp paired-end-protocol. After trimming and merging of paired-end reads, the taxonomic assignment was performed using the Bayesian LCA-based classification method (Gao *et al.* 2017) against the NCBI 16S microbial database.

Alpha diversity showed no major variation among the samples although species richness differed significantly between abomasum and colon, independently of resistance genotype. For beta diversity analysis, Principle Coordinates Analysis (PCoA) was carried out using the weighted UniFrac metric, and it exposed a specific clustering in the duodenum. The Linear Discriminant Analysis (LDA) Effect Size (LEfSE) algorithm was used to compare bacterial abundances between the groups. In the duodenum, there was a significant clustering of bacterial populations for the resistant and susceptible groups. The LEfSE analysis indicated that the most abundant genera in the R group were *Solobacterium*, *Ruminococcus* and *Mogibacterium* whereas, in the L group, *Succiniclasticum*, *Aminipila*, *Butyrivibrio* and *Saccharofermentans* were most abundant (Figure 1).

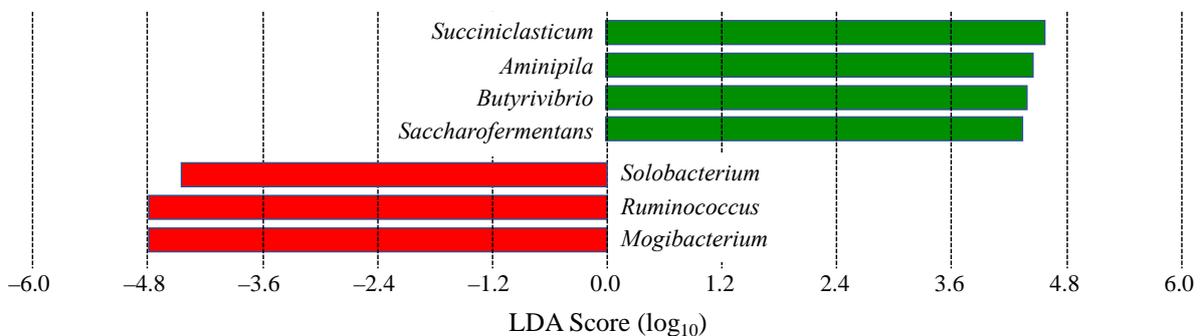


Figure 1. Linear Discriminant Analysis comparing duodenal populations of bacteria in worm-resistant (R; red) and worm susceptible (S; green) Merino sheep.

These observations show that the microbial composition of the sheep duodenum is affected by breeding for resistance to helminths, especially the genera *Succiniclasticum*, *Aminipila*, *Butyrivibrio* and *Saccharofermentans*. We therefore suggest that these changes play an important role in the expression of the low WEC phenotype. It is plausible that short-chain fatty acids produced by the favoured bacterial species help to reduce the parasite load in the resistant sheep. It is worth noting that there is a relationship between helminth infection and butyrate-producing bacteria in goats (Li *et al.* 2016). Interestingly, in the susceptible sheep, *Mogibacterium* is the only non-fermentative entirely asaccharolytic genus. These findings provide valuable insights into the processes that affected by, and perhaps responsible for resistance and susceptibility to helminths.

References

- Greeff JC and Karlsson LJE (2020) *Animal Production Science* doi: 10.1071/AN19368.
- Mamun MAA, Sandeman M, Rayment P, Brook-Carter P, Scholes E, Kasinadhuni N, Piedrafita and Greenhill AR (2020) *Animal Microbiome* 2, 1-14.
- Gao X, Lin H, Revanna K and Dong Q (2017) *BMC Bioinformatics* 18, 247.
- Li RW, Li W, Sun J, Yu P, Baldwin RL and Urban JF (2016) *Scientific Reports* 6, 1–10.