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FAECAL MICROBIOTA OF ANGUS CATTLE WITH DIVERGENT IMMUNE COMPETENCE

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SUMMARY

Microorganisms inhabiting the gut (gut microbiota) have been shown to influence immune responsiveness of the host in a variety of species. It has also been discovered that specific species of gut microbiota may contribute to immunity in multibreed cattle. In this study, faecal samples were obtained from Angus cattle that were concurrently phenotyped for cell-mediated and antibody-mediated immune responsiveness (IR) at weaning. Both IR phenotypes, and an ImmuneDex score, were calculated and used to identify high, medium and low IR cohorts (n=20/group). 16s rRNA gene sequence data was used to infer the relative abundances of different phyla in the sampled animals. A total of 6 phyla were found to significantly differ in relative abundances for at least one of the IR phenotypes. Of these, Bacteroidota, Euryarchaeota and Proteobacteria may be biologically relevant due to their relationship with gut health and disease.

INTRODUCTION

Gut microbiota play an important role in modulating host immune responses. Specifically in livestock, recent studies indicate that host immune responsiveness is linked with gut microbiota profiles in both pigs and multibreed cattle (Fan *et al*. 2021; Ramayo-Caldas *et al*. 2021). Gut microbiota have also been reported to differ between different cattle breeds (Fan *et al*. 2021), and there is evidence indicating the relative abundance of some groups of gut microorganisms may be heritable (Fan *et al*. 2021). Therefore, the aim of this study was to determine whether there were significant differences in the faecal microbiota profiles of Angus cattle cohorts with high, medium, or low immune responsiveness. This could be used to develop a better understanding of microbial profiles and specific gut microorganisms differing between IR cohorts and could further lead to the development of a variety of selection or intervention tools that makes Angus production more profitable.

MATERIALS AND METHODS

Rectal faecal samples were collected from 444 Angus weaners (6months of age) run on pasture at Charles Sturt University and Talooby farms over 2021 and 2022. Rectal faecal samples were put on dry ice immediately after collection and stored in the laboratory at -20°C until further processing.

Measurement of immune responsiveness. During rectal faecal sampling, all weaners were concurrently phenotyped for cell-mediated and antibody-mediated immune responsiveness. A

measurement representing a combined cell-mediated and antibody-mediated response, known as ImmuneDEX, was also estimated as previously described (Reverter *et al*. 2021).

DNA extractions and 16s rRNA gene sequencing. Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA) was used to extract genomic DNA from faecal samples as per manufacturer's instructions. NanoDrop 2000 Spectrophotometer (Thermo Scientific, Australia) was used to determine final yield and quality of extracted DNA. Finally, samples were subjected to paired end 16s rRNA gene sequencing at the Novogene sequencing facility in Singapore.

Statistical analysis. All IR phenotypes were calculated via linear regression after accounting for contemporary groups (based on herd ID, calf year of birth, cohort, sex, dam year of birth), age at measurement and weaning weight. These phenotypes were subsequently transformed into z-scores, and 20 animals with the highest, lowest, or z-scores closest to zero, were classified into high, low and medium IR cohorts respectively. Sequence data was used to create relative abundance graphs in R (using packages ggplot2 and ggpubr), and analysis was limited to the taxonomic level of phyla due to space limitations. Statistical analyses was performed using MANOVA in R to identify significant differences in relative abundances of the top 15 most abundant phyla between different IR cohorts.

RESULTS AND DISCUSSION

The average z-scores along with standard deviations are presented in Table 1. While there were some animals shared between high, medium and low cohorts of different IR phenotypes, a majority of animals were different. For instance, only one animal was common between the antibody-mediated and cell-mediated IR phenotypes in the high IR cohort. On the other hand, ImmuneDEX which is strongly correlated with the other two IR phenotypes, had more animals shared in common in its high cohort when compared to the high antibody-mediated IR cohort (8 animals) and the high cell-mediated IR cohort (7 animals).

Table 1. Z-scores (Mean ± Standard deviation) for high (n=20), medium (n=20) and low (n=20) IR cohorts for each of the three IR phenotypes

IR Cohorts	IR phenotypes		
	Antibody-mediated	*Cell-mediated*	*ImmuneDEX*
High	2.084 ± 0.21	2.285 ± 0.31	2.736 ± 0.49
Medium	0.008 ± 0.04	-0.006 ± 0.03	-0.003 ± 0.02
Low	-1.969 ± 0.21	-1.909 ± 0.26	-2.452 ± 0.35

The average relative abundances of different phyla, inferred based on 16s rRNA gene sequences (Figure 1), revealed Firmicutes and Bacteroidota to be the two most abundant phyla, which is consistent with scientific literature (Fan *et al*. 2021). Together these phyla account for ~ 90% of all microorganisms represented in the faecal samples. Analysis of the relative abundance data also revealed several significant differences between phyla of high, medium and low cohorts of different IR phenotypes. These differences have been presented in Table 2.

Bacteroidota was the only phylum whose relative abundance was found to significantly differ in the antibody-mediated IR phenotype. Bacteroidota have been previously reported to contribute to the development of the immune system, and to anti-inflammatory responses (Gibiino *et al*. 2018). They have also been linked to the activation of Th1 systemic immune responses, as well as stimulation of B cells (Ivanov *et al*. 2008).

The cell-mediated IR phenotype had two phyla that were found to significantly differ in terms of relative abundance, Euryarchaeota and Fusobacteriota. Existing evidence suggests Euryarchaeota are comprised of methanogenic species existing in the gut. In humans, it is possible that these methanogens have either a direct or indirect contribution to the development of gastrointestinal disorders and therefore, can adversely impact host health (Horz *et al.* 2010).

In the ImmuneDEX IR phenotype, Proteobacteria, Fusobacteriota and Acidobacteriota were found to differ significantly. Proteobacteria have previously been reported to increase in abundance in the gut of individuals with a compromised immune system and could potentially be indicative of a diseased state (Shin *et al*. 2015).

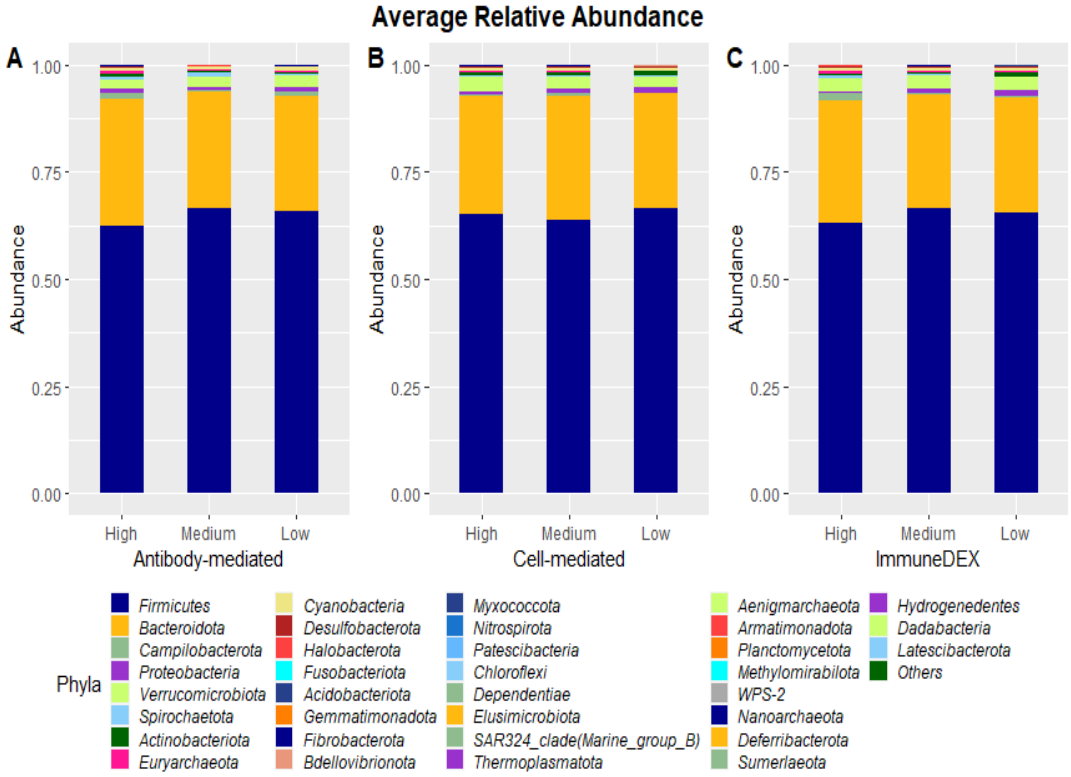

Figure 1. Phylum-level faecal microbiota assortment. Bar chart representing the average relative abundance of all bacterial ASVs taxonomically classified for A) antibody-mediated B) cell-mediated and C) ImmuneDEX, high, medium and low cohorts

Table 2. Represents the phyla that significantly differed between high, medium and low cohorts for antibody-mediated, cell-mediated and ImmuneDEX phenotypes

Antibody-mediated (P=0.05)	
Phyla	*P-value*
Bacteroidota	0.044*
Cell-mediated (P=0.05)	
Phyla	*P-value*
Euryarchaeota	0.035*
Fusobacteriota	0.040*
ImmuneDEX (P=0.05)	
Phyla	*P-value*
Proteobacteria	0.022*
Fusobacteriota	0.002*
Acidobacteriota	0.049*

Note. * Indicates a significant value (P=0.05)

CONCLUSION

Overall, 6 phyla out of the top 15 phyla, in terms of relative abundances, were found to significantly differ between the high, medium and low cohorts of the three IR phenotypes. The presence of Bacteroidota in the antibody-mediated phenotype, Euryarchaeota in the cell-mediated phenotype and of Proteobacteria in the ImmuneDEX phenotype, could be biologically relevant and warrants further in-depth investigation including investigating the heritability of these abundances and how much variation is explained by the host. Characterising microbiome-based signatures of different IR states could help identify at-risk animals and afford opportunities for early intervention that could improve animal health, welfare and productivity.

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