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**ASPECTS OF DIETARY MANAGEMENT AND DYNAMICS
OF THE FAECAL MICROBIOTA OF HORSES AND PONIES
(*Equus caballus*) IN NEW ZEALAND**

A thesis presented in partial fulfilment of the requirements for the
degree of

DOCTOR OF PHILOSOPHY
in
Veterinary Science

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*This thesis is dedicated to the wonderful people and animals who
have taught me so much about life...
especially to beloved Ricki*



ABSTRACT

The aim of this thesis was to explore aspects of the dietary management of horses and ponies in New Zealand, and to investigate the association between dietary management and faecal microbiota. To achieve this aim, a series of observational and intervention studies were conducted.

The first two studies were cross-sectional surveys of feeding, health and management practices, which showed that most horses and ponies in New Zealand were managed continuously on pasture all year round, with no seasonal differences in the hours allowed for grazing. In addition to pasture, many owners fed their animals a combination of premixed feeds, cereals (oats) and conserved forages. Most horses and ponies kept on pasture were reported to be healthy. Among nutrition-related health issues reported by the owner, obesity, colic, laminitis and grass staggers were most commonly reported (12-14%). Using a standard body condition scoring chart, 22% of owners indicated that their horse or pony was overweight (scored ≥ 7 on a 1-9 scale). Horses and ponies kept on pasture maintained body weight and a higher body condition (median score of 6 on a 1-9 scale) through spring and autumn. A high proportion of pony breeds were observed in this population, and these ponies remained 'fat' despite the seasonal fluctuation in the quantity and quality of pasture. Owners tended to underestimate the body condition of their horses, especially ponies, and this finding indicated why a higher percentage of overweight animals may be present in the Pony Club population.

The next two studies were observational investigations that characterised the faecal microbiota of forage-fed horses. The faecal microbiota in a cohort of yearling Thoroughbred horses that were abruptly transitioned from an ensiled chopped forage-based diet to pasture was diet-specific and responded rapidly to dietary change within four days. The faecal microbiota profile was dominated by two phyla, Firmicutes and Bacteroidetes, which comprised of several bacterial genera. The abundance of bacterial genera fluctuated over the three-week observation period, when kept at pasture. Similarly, the faecal microbiota of a cohort of mature adult Thoroughbred and Standardbred horses kept on pasture was diet-specific. The abundances of the bacterial genera were influenced by the nutrient composition of the pasture, which was also correlated with seasonal changes in climate (rainfall and temperature) over the one year observation period. This latter finding indicated that the fluctuations observed in the previous study may also be due to changes in pasture composition.

The inclusion of hay in the diet appeared to buffer the changes occurring in the faecal microbiota as a result of the seasonal fluctuations in pasture composition, but there was also a large degree of variation between individual horses.

The final study was a randomised controlled trial using adult Thoroughbred horses that were kept in loose boxes and fed four forage-based diets. The first phase of the trial identified that the mean retention time of digesta was associated with the dry matter intake of the feed consumed. There was a significant difference in the quantity of feed consumed by individual horses, which appeared to be driven by the moisture content in the forage diets. The second phase of the trial showed that the population of the faecal microbiota was resilient following abrupt dietary transition between four forage-based diets. These findings indicated why the horses in the previous study may have maintained body weight and condition, despite the seasonal fluctuation in the quantity and quality of pasture.

This thesis highlights the complexity of the equine faecal microbiota, and demonstrates that the relationship of dietary dry matter intake and mean retention time of digesta in the gastrointestinal tract influences the population dynamics of the faecal bacterial community.

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Karlette Anne Fernandes

Palmerston North, New Zealand

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LIST OF ABBREVIATIONS

| | |
|-----------------|---|
| ADF | Acid detergent fibre |
| ANOSIM | Analysis of Similarity |
| ANOVA | Analysis of Variance |
| BCS | Body condition score |
| BW | Body weight |
| BWA | Burrows Wheeler aligner |
| CFU | Colony forming units |
| CH ₄ | Methane |
| CHO | Carbohydrates |
| CNS | Cresty neck score |
| DE | Digestible energy |
| DM | Dry matter |
| DMI | Dry matter intake |
| ECS | Equine Cushing's disease |
| eHGM | Equine hindgut microbiome |
| EMS | Equine metabolic syndrome |
| ESNZ | Equestrian Sports New Zealand |
| F:B | Firmicutes: Bacteroidetes |
| FE | FiberEzy® |
| FW | Faecal weight |
| GE | Gross energy |
| GI | Gastrointestinal |
| HNF | High Nutritional Fiber |
| IQR | Interquartile range |
| IR | Insulin resistance |
| MEGA | Molecular Evolutionary Genetics Analysis |
| MJ | Megajoules |
| MRT | Mean retention time |
| MUAEC | Massey University Animal Ethics Committee |
| NC | Neck circumference |
| NCBI | National Centre for Biotechnology Information |

| | |
|-------|---|
| NDF | Neutral detergent fibre |
| NFC | Non-fibre carbohydrates |
| NSC | Non-structural carbohydrates |
| NSC | Non-structural carbohydrates |
| NZCAC | New Zealand Companion Animal Council |
| NZPCA | New Zealand Pony Clubs Association |
| OR | Odds ratio |
| OTU | Operational taxonomic units |
| PAM | Post-administration of markers |
| PAST | Paleontological Statistics |
| PC | Pony club |
| PCoA | Principal coordinates analysis |
| PCR | Polymerase chain reaction |
| QIIME | Quantitative Insights into Microbial Ecology |
| SD | Standard deviation |
| SRA | Sequence read archives |
| TDN | Total digestible nutrients |
| UPGMA | Unweighted Pair Group Method with Arithmetic Mean |
| USDA | United States Department of Agriculture |
| VFA | Volatile fatty acids |
| WSC | Water soluble carbohydrates |