Faeces: A model for hindgut function?

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Introduction The horse is a non-ruminating monogastric herbivore that has evolved over millions of years to a grazing and browsing existence, in which it has adapted to graze on high fibre, low energy fodder by the aid of a complex microbial community (Milinovich *et al*., 2006). However, domestication of the horse has led to this natural feeding pattern being disturbed, and consequently gastrointestinal disease is the single most important cause of mortality in the domestic horse (Daly *et al*., 2001). Nonetheless, despite its importance, the microbial community of the equine hindgut has received relatively little attention. An understanding of the microbiology of the equine gastrointestinal tract (GIT) is essential in improving our understanding of digestive processes, and for the prevention and treatment of disease involving the GIT. While microbial diversity has an important role to play in hindgut function and disease, its effect on the ability to degrade certain feedstuffs is of equal importance. Therefore, the capacity to assess the effects of feedstuffs on the large intestinal environment is essential to further our understanding of normal and disease processes within the GIT of the horse. However, many studies investigating microbial diversity and fermentation characteristics within the equine hindgut typically used animals specifically euthanased for the purpose, or surgically-modified. This is expensive, and highly invasive, and there is an urgent requirement to replace and refine these methods, with more cost-effective, welfare-friendly alternatives.

Assessment of microbial populations in the hindgut of the horse Current knowledge of gut microbial ecology and diversity is almost exclusively based on the use of classic culture-based methods that are often laborious, time consuming and may only recover a fraction of the microbial diversity present within the gut (Daly *et al*., 2001). However, advanced modern molecular methods, such as real-time semi-quantitative PCR (Q-PCR), are culture-independent tools for accurate and sensitive quantification of individual bacterial species, as well as total bacterial numbers (Nadkarni *et al*., 2002). Published data on the identification/quantification of intestinal bacteria using Q-PCR technology, which is a more accurate and sensitive alternative to conventional end-point PCR-based methodologies has been applied to study diet-dependent shifts in the bacterial populations of the rumen (Tajima *et al*., 2001), infant gut (Haarman and Knol, 2005) and, more recently, to the hindgut of the horse (Hastie *et al*., 2008). The latter study was conducted to determine the abundance of candidate cellulolytic (*R. flavefaciens*; *F. succinogenes*) and non-cellulolytic (*S. bovis*) bacteria in frozen and lyophilised lumen contents from the caecum, ventral and dorsal colon, and rectum of healthy horses. Results showed frozen and lyophilised samples to contain similar levels of *R. flavefaciens*, *F. succinogenes* and *S. bovis* relative to total bacterial load in luminal contents obtained from the dorsal colon and rectum, indicating that, similar to other monogastric animals (Whitehead and Cotta, 1993), equine faecal material could reflect the microbiological characteristics of the distal colon. Furthermore, data from the frozen luminal contents indicated similarities between the three bacteria in the ventral colon, dorsal colon and rectum, potentially allowing faeces to be used as a model for the whole colon. This would subsequently allow faeces to act as a model for the distal colon facilitating accurate determination of changes in gut micro-flora without the need for surgically modified animals or the use of slaughter material, which allows for no information on the animal's health or dietary management.

Assessment of fermentation in the hindgut of the horse In recent years the *in vitro* gas production technique of Theodorou *et al*. (1994) has been used to assess the effects of feedstuffs on large intestinal environment, using caecal fluid (McLean *et al*., 1997) and more recently faeces (Murray *et al*., 2005) as the source of inocula. This technique has also been used to assess the fermentative capacity of equine faecal inocula obtained from individual ponies, as an indicator of hindgut microbial activity (Murray *et al*., 2006). It has been hypothesised that there may be differences in fermentative capacity between individual horses as a result of microbial diversity, and that these differences may be particularly evident in animals with a history of diseases affecting the GIT, such as laminitis. However, recent results (Murray *et al*., 2009) show no difference between the ability of faecal inocula obtained from ponies with or without a history of laminitis to ferment grass hay, starch or inulin. Nevertheless, this work needs to be expanded to further validate the use of faecal inocula in the gas production technique as a model of hindgut function. In particular, there is a need to determine inter-animal variability in terms of hindgut regional variation in inocula source.

Conclusion By establishing a model of hindgut function using non-invasive techniques, further research can explore the role of key bacteria in different stages of gastrointestinal disease, and not just at the terminal stages following euthanasia. If a conclusive link can be established in healthy horses using faecal material to give an indication of bacterial community structure, then faecal material could potentially become a non-invasive tool to accurately monitor changes in the colonic bacterial populations in response to diet and other environmental factors, and allow for the accurate measurement of potential disease-causing bacteria in the colon.

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