The Hindgut Microbiome of Grazing Horses

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Abstract. The hindgut microbiome plays an essential role in horses consuming forage-based diets high in fiber, such as pasture. The equine hindgut harbors a large microbial community that ferments dietary fiber and produces by-products which provide a substantial portion of daily energy requirements. Despite the importance in nutrition and health, research evaluating the hindgut microbiome of grazing horses is relatively limited. Grazing horse microbiome research has primarily focused on inter-diet comparisons with mixed diets including concentrates as well as with other forms of forage. Recent research has begun to explore responses of the gut microbiome to different pasture forage species and relationships with horse metabolism. Further research is needed to unravel the complex relationships between pasture management practices, impacts on the hindgut microbiome, and horse health outcomes. The objective of this presentation will be to discuss recent research and highlight future research needs and opportunities related to the microbiome of the grazing horse.

Introduction

Horses are classified as hindgut fermenters. The main site of digestion and absorption of dietary sugars, starches, fats, and protein is the small intestine. However, like other mammalian species, horses do not produce enzymes capable of breaking down more complex carbohydrates such as fructans, cellulose, and hemicellulose. Rather, fructans and dietary fibers pass into the hindgut and are subject to microbial fermentation. While the primary function of the hindgut microbiota is to enable the horse to extract energy from these more complex, recalcitrant plant components, hindgut bacteria are also capable of fermenting any nutrients escaping digestion in the foregut (i.e. sugars, starches, or proteins). Given its essential role in fiber degradation and the high-fiber content of pasture forages, the hindgut microbiota is of central importance in the nutrition and health of grazing horses. Grazing horse microbiome research has primarily focused on inter-diet comparisons with concentrates or other forages. Seasonal and environmental associations as well as relationships with horse metabolism have also been explored. Research in this field is thus far limited, and future research is needed to better understand adaptations of the hindgut microbiome in response to pasture management practices as well the role of the microbiome in health outcomes of grazing horses. The objective of this presentation will be to discuss recent research and highlight future research needs and opportunities related to the microbiome of the grazing horse.

Inter-Diet Comparisons

Diet is considered the dominant factor affecting community structure of gut microbiota across animal species including the horse. In horses, inter-diet comparisons have been conducted to evaluate the hindgut microbiome of horses adapted to diets of varying composition as well as responses to transitions between diets. Studies evaluating inter-diet comparisons have largely characterized differences in the hindgut microbiota of horses consuming concentrate vs. concentrate or concentrate vs. forage diets, with comparatively few studies focused on forages alone (Julliand and Grimm 2017). Equine microbiome research in pasture-fed horses is particularly limited.

Differences in microbial community structure, species composition, and function have been documented in horses fed high-concentrate versus high-forage diets (Garber et al. 2020a). In general, the microbiomes of horses fed higher-forage diets (in comparison to higher concentrate diets) are characterized by greater α-diversity and temporal stability; greater prevalence of cellulolytic bacteria; lesser prevalence of amylolytic and lactate-utilizing bacteria; and greater butyrate and acetate concentrations with less propionate and lactate (Grimm et al. 2017; Garber et al., 2020a).While the majority of these studies have

utilized conserved forages (hay or silage), inter-diet comparisons between the microbiome of horses grazing pasture forage and horses fed mixed diets including concentrates have similarly demonstrated greater α-diversity in the hindgut microbiome of grazing horses (Fernandes et al. 2014). Daly et al. (2012) also reported greater relative abundance of *Fibrobacter* and *Ruminococcaceae*, with lesser abundance of *Bacillus, Streptococcus,* and *Lactobacillus* and lower concentrations of lactic acid in pasture-fed horses than horses fed mixed diets.

Few studies have characterized the grazing horse microbiome in comparison to other forage types or across forage species. Zhu et al. (2021) found greater α-diversity in horses maintained on pasture in comparison to horses fed either silage or hay, as well as differences in β-diversity between forages. Microbial community composition was also impacted by forage type, with abundance of *Streptococcaceae* lowest in pastured horses (Zhu et al. 2021). Differences in fecal microbial structure and composition have also been identified in horses grazing warm- versus cool-season grasses as well as in comparison to a standardized hay diet (Weinert-Nelson et al. in review).

Given the implications for gastrointestinal health, responses of the hindgut microbiota to abrupt dietary change is also of interest for equine feeding management. Rapid changes in the hindgut microbiome have been reported following abrupt changes between concentrates or abrupt inclusion/elimination of supplemental concentrates in horses maintained on hay or pasture (Garber et al. 2020a). Comparatively few studies have investigated longitudinal shifts in the hindgut or fecal microbiome of horses during transitions between all-forage diets; however, results suggest more subtle changes in comparison to diets including concentrate (Garber et al. 2020a). Garber et al. (2020a) found no significant changes in fecal α-diversity (either species richness or evenness) over a 14-d transition between hay and pasture, but did identify broad phylum-level changes for *Firmicutes* and *Bacteroidetes* as well as various genera enriched on specific days of the transition periods. In contrast, Fernandes et al. (2021a) documented resilience of the equine hindgut microbiota in response to transition between pasture forage and silage, with microbial communities exhibiting stability after 96 hours. To the authors' knowledge, only one study has documented the impacts of grazing differing pasture forages on the equine microbiome. Weinert-Nelson et al. (2022) reported that equine fecal microbial community structure and composition were largely stable across transitions between warm-season grass and cool-season grass pasture. Research is needed to expand on this work across additional grass species/varieties and to include assessments of horses grazing legume forages.

Relationships Between Forage Nutrients, the Microbiome, and Equine Metabolism

Research conducted in mouse models has demonstrated that changes in diet influence host metabolism in a microbiome-dependent manner (Zhao et al. 2018). While associations between the equine hindgut microbiota and metabolic health have been explored (Fitzgerald et al. 2020), there is a lack of information on the interplay between forage nutrients, the hindgut microbiome, and metabolism of grazing horses. Fernandes et al. (2021) observed correlations between pasture nutrient composition and several unclassified bacterial genera. Weinert-Nelson et al. (in review) demonstrated that non-fiber nutrient fractions including CP and non-structural carbohydrates (NSC) exerted the greatest influence on bacterial species composition in the microbiomes of grazing horses. Weinert-Nelson et al. (in review) also found that bacteria such as *Akkermansia* and *Clostridium butyricum* were positively correlated with CP and negatively correlated with NSC; *C. butyricum* was also negatively correlated with peak plasma glucose in response to administration of an oral sugar test. However, additional research is needed to further unravel the complex relationships between pasture forage nutrients, impacts on the hindgut microbiome, and metabolic responses in grazing horses.

Seasonal Fluctuations and Climate

Equine microbiome researchers have begun to explore the impacts of season and climate on the hindgut microbiome of the grazing horse. Salem et al. (2018) reported seasonal changes in fecal microbial community of pastured horses and found that weather variables including temperature and precipitation

were associated with compositional changes. Fluctuations in bacterial diversity and relative abundance across seasons and relationships with climatic conditions were also subsequently documented by Fernandes et al. (2021). However, seasonal controls were not implemented in these prior studies, leaving potential implications of seasonal changes confounded by inherent differences in pasture forage quality and availability.

Future Directions

Overall, grazing horse microbiome research is limited. Impacts of management systems and stocking methods, common pasture management practices, pasture quality, and effects of commonly utilized supplemental feeding strategies are unknown. An expansion of existing research is required to determine responses of the hindgut microbiota across pastures with varying botanical composition as well as to evaluate geographical and climatic influences. Implications across physiological stages should be evaluated including in broodmares and foals. Additional research is also needed to better define the role of the microbiome in health outcomes of pasture-managed horses.

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