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Original Research

Effects of Feeding Management on the Equine Cecal Microbiota

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ABSTRACT

The effects of meal size and frequency on equine cecal microbiota are not well documented. We hypothesized that changes in feeding management (including differing meal size and frequency) would alter the equine cecal microbiota. Cecally cannulated horses (n = 6) were utilized in a 3 × 3 Latin square where they received group pasture turnout daily and were stalled overnight in box stalls (3 × 4 m). Treatment diets consisted of Strategy® concentrate and were as follows: A = one meal, 2.72 kg, 6 AM; B = two meals, 1.36 kg/meal, 6 AM and 4 PM; and C = three meals, 0.91 kg/meal, 6 AM, 12 PM, and 4 PM. Treatment periods consisted of 8 days of acclimation followed by 3 days of collection. All horses received ad libitum access to water, a white salt block, and 3 kg of mixed alfalfa/grass hay offered overnight. Cecal samples were sequenced using 16S rRNA gene-based Illumina technology. Data were analyzed using QIIME 1.8.0 and Proc MIXED of SAS. Weighted principal coordinates analysis values indicated that feeding management impacted cecal microbiota composition with horses fed one large meal having different microbial community than those fed three smaller meals throughout the day ($P = .028$). Furthermore, treatment affected ($P < .05$) *Prevotella, YRC22, Lactobacillus, Streptococcus, Coprococcus*, and *Phascolarctobacterium*. These data demonstrate that feeding a single large bolus meal affects both abundance and composition of the cecal microbiota. Further research is necessary to understand the metabolic and/or health implications related to changes the equine cecal microbiota.

1. Introduction

Meal size and frequency are components of feeding management programs that are continually evaluated and modified by nutritionists and veterinarians. However, studies have demonstrated that owners consistently overfeed their horses and that 60% of owners have no discernable nutrition management plan [1]. What is perhaps even more startling is that only one third of all horses receive the correct amount of forage in their diet and that a large majority (71%) of horses are still being fed based on the owner's visual assessment of concentrate (i.e., scoop, coffee can, or handfuls) rather than a specific weight [2]. In light of these surprising facts, it seems clear that better information and better owner education programs are needed to implement much needed improvements to dietary management strategies. More studies aimed at

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understanding the microbiological impacts of those feeding errors may provide critical information to assist veterinarians and nutritionists in educating horse owners.

The gastrointestinal microbiota of the equine is intrinsically connected to overall horse health. Horse owners today commonly utilize commercial rations to supply additional energy for the demands associated with performance, reproduction, and lactation. The natural grazing cycle, which has been documented as 10 to 15 h/d [3], is artificially interrupted by stalling or other performance-related activities. Unfortunately, the interruption of the grazing cycle coupled with the use of high-energy concentrates in equine rations has been linked to inadequate small intestinal digestion and has been reported to result in microbial perturbations, decreased intestinal pH, a disruption of gut motility, and an increased risk of laminitis and colic [4–7].

Meal size and frequency impacts gastric emptying [3], serum leptin [8], blood glucose [9] concentrations, and stable vices (cribbing, weaving, etc.) in horses [10]. Additionally, studies conducted in mice have shown an effect on the overall gut microbiome [11]. Although recent access to next-generation sequencing techniques has facilitated greater characterization and study of the microbial communities in the equine gut [12–18], most studies have focused on feces so relatively little is known about the effect of meal size or frequency on the equine cecal microbiota. The purpose of this study was to test the hypothesis that changing meal size and frequency would result in a change of the equine cecal microbiota.

2. Materials and Methods

2.1. Animals and Diets

Southern Illinois University Institutional Animal Care and Use Committee approval was obtained prior to the initiation of this study (#13-070). Six stock-type surgically cannulated horses [19] were utilized in a replicated 3 × 3 Latin square design. Mean body weight (BW) and physical characteristics are shown in Table 1. Trained personnel weighed the horses once weekly using a livestock scale (Horse Stock Platform Scale, Priefert, Mount Pleasant, TX) and was assessed body condition score (BCS) on days 0 and 11 of each period, respectively. All horses maintained BCS and demonstrated no significant weight loss (defined as > 22.72 kg) throughout the study.

Horses were randomly assigned to one of three treatment groups using a single pelleted concentrate ration (Strategy®; Purina Animal Nutrition, St. Louis, MO) described as follows: treatment A = single meal of 2.72 kg pelleted concentrate ration at 6 AM once daily; treatment B = two meals of 1.36 kg pelleted concentrate ration at 6 AM and 4 PM daily; and treatment C = three meals of 0.91 kg each at 6 AM, 12 PM, and 4 PM daily. Concentrate ingredients were as follows: wheat middlings, ground soybean hulls, cane molasses, suncured alfalfa, corn germ meal, ground corn, soybean oil, calcium carbonate, dehulled soybean meal, salt, lignin sulfonate, calcium propionate (a preservative), L-lysine, DL-methionine, vitamin E supplement, colored with iron oxide, vitamin D3 supplement, vitamin A supplement, zinc oxide, copper sulfate, choline chloride, natural anise flavor, artificial flavors, vitamin B-12 supplement, riboflavin supplement, calcium pantothenate, niacin supplement, calcium iodate, magnesium oxide, cobalt carbonate, ferrous carbonate, sodium selenite, manganous oxide, saccharin sodium, and maltodextrin. All horses were allowed 8 days of acclimation prior to a 3-day collection phase during each treatment period. Daily, all horses were allowed 6 hours (approximately 8 AM–2 PM) of group pasture turnout, to provide appropriate social interaction and enrichment opportunities into a 12-acre field consisting of approximately 60% K31 tall fescue, 10% orchard grass, and 30% volunteer white clover. The pasture had been rested for 4 weeks prior to the initiation of the study. Based on a carrying capacity of 3.3 animal units per month and the utilization of a grazing stick, the number of grazing days estimated for this pasture was 15 days per acre. Horses were stalled overnight in identical 3 × 4 m stalls with ad libitum access to water along with 3 kg of mixed grass/alfalfa hay. Feed samples for pasture, hay, and concentrate were collected during each period and were pooled for analysis (Table 2).

Table 1
Physical characteristics of cecally cannulated horses utilized in feeding management study.

ID	Breed	Age (y)	BW (mean)	BCS (mean)	Gender
Horse 1	Thoroughbred	9	551	4.5	Mare
Horse 2	Thoroughbred	11	513	4.5	Mare
Horse 3	Appendix	7	502	5	Mare
Horse 4	Quarter Horse	4	508	5.5	Mare
Horse 5	Quarter Horse	6	557	5	Gelding
Horse 6	Quarter Horse	7	484	4.5	Mare

Abbreviations: BCS, body condition score; BW, body weight.

Table 2
Nutrient analysis[a,b] of hay, pasture, and concentrate ration consumed by cecally cannulated horses daily.

Nutrient Content (%)	Diet		
	Fescue Pasture	Hay	Strategy Grain[c]
Crude protein	13.6	16.9	16.8
Lysine	0.47	0.59	0
Acid detergent fiber	36.4	38.8	17.2
Neutral detergent fiber	58.7	56.6	35.9
Water soluble carbohydrate	7.1	8.6	9.5
Ethanol soluble carbohydrate	5.1	5.9	5.6
Starch	3.0	2.0	15.7
Nonfiber carbohydrate	18.0	17.3	37.6
Calcium	0.53	1.00	1.32
Phosphorus	0.31	0.31	0.81
Magnesium	0	0	0.39
Potassium	0	0	1.28
Sodium	0	0	0.267
Nutrient content (ppm)			
Iron	0	0	697
Zinc	0	0	366
Copper	0	0	93
Manganese	0	0	245
Molybdenum	0	0	1.2
Digestible energy (Mcal/kg)	2.19	2.19	2.97

[a] Analysis performed by Equi-Analytical, Ithaca, NY.
[b] Expressed on dry matter basis.
[c] Purina Animal Nutrition, St. Louis, MO.