Review Article

The Impact of Diet on the Hindgut Microbiome

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A B S T R A C T

In horses, dietary components that are not digested by host enzymes in the foregut pass unchanged through to the hindgut where they have a profound effect on the microbiome. Epidemiologic studies have given evidence that important equine health issues could result from dysbiosis in the hindgut ecosystem as a result of dietary factors. This review presents an outline of plant carbohydrates degradation by the equine hindgut microbiome. Then, data are summarized from studies assessing the variations of the microbiome occurring in the hindgut under different dietary changes. In the literature, most studies examined the impact of abrupt changes and changes between high-starch versus high-fiber diets, while the effect of feed form and processing or feeding frequency was rarely explored. Finally, the representativeness of the fecal microbiome changes is compared to the hindgut changes when horses are submitted to dietary changes.

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1. Introduction

In horses like in other species, diet has a profound effect on the microbiome of the hindgut. Dietary components that are not digested by host enzymes in the foregut pass unchanged through to the hindgut and deliver a variety of growth-promoting and growth-inhibiting factors which influence the balance between species specialized for the fermentation of different substrates within the microbial community. Non-digestible plant cell wall polysaccharides provide substrates for the growth of fibrolytic microorganisms, which is essential to the host, as they in turn provide energy via the end products of their metabolism, especially short-chain fatty acids (SCFAs).

Epidemiologic studies have given evidence that important equine health issues such as colic [1–7] and laminitis [8,9] could result from dysbiosis in the hindgut ecosystem as a result of dietary factors. Primary data also showed substantial alterations in the hindgut bacteria of horses submitted to abrupt ingestion of concentrate, provoking an acute form of laminitis [10].

The direct study of the hindgut microbiota composition as well as its activity provides a clear picture of the state of the microbial ecosystem balance. Direct studies have been conducted experimentally using cecal and colonic contents collected from fistulated or euthanized horses, but which is not feasible in the field. Using other materials such as feces could provide an easily collected alternative thus enabling the observation of dietary-related variations of the hindgut microbial ecosystem. Fecal data could provide information regarding microbial balance and efficiency of fiber degradation and thus giving insight to prevent the incidence of colic or laminitis.

Along this review, we studied only information that looked specifically at equine. After an outline of plant carbohydrate degraders, we summarized data from studies assessing the variations of the microbiome occurring in the hindgut under different dietary changes. Finally, we discussed the representativeness of the fecal microbiome changes compared to the hindgut ones when horses are submitted to dietary changes.

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2. The Hindgut Microbiome and Plant Carbohydrate Degraders

There would be no horse without hindgut microorganisms! Indeed, cell wall carbohydrates (including cellulose, hemicelluloses, and pectins) that represent from 35% to 60% on average in the equine diet contain bonds that are resistant to the horse's hydrolytic enzymes. Only microbial enzymes can break them down in the hindgut, which offers a propitious environment with ideal physicochemical characteristics for microorganisms' growth and fermentative activity.

Dietary starch and water-soluble carbohydrate (WSC) represent from about 10% to 40% of the daily ration [11]. A fraction of this carbohydrate fraction may not be fully digested in the foregut, either due to starch granule structure or to a limited capacity of the amylase or a lack of fructanase in the small intestinal.

In the hindgut plant, carbohydrate degradation involves two steps: first the hydrolysis of complex polysaccharides and second the fermentation of the simple sugars (Fig. 1).

2.1. Complex Polysaccharides Utilizers

The first step of the complex polysaccharides degradation process consists of the attachment of microorganisms to plant cell walls. This adhesion directs concentrated enzymes to the substrates, ensuring hydrolysis of polysaccharides. Enzymes involved in plant cell wall and starch degradation were shown to originate mainly from bacteria bound to particles both in the equine cecum and colon content, rather than from bacteria present in the liquid phase [12].

Fungal species of *Piromyces* genus and bacterial species of the *Ruminococcus* and *Fibrobacter* genera have been identified as major fibrolytic microorganisms responsible for plant cell wall degradation in the equine hindgut. Further detail of those microorganisms involved in dietary plant cell walls degradation and more generally of the equine hindgut microbiome has been published in a recent review [13].

The main amylolytic bacteria identified in the equine hindgut belong to the *Streptococcus* and *Lactobacillus* genera. Some strains have been isolated: *Streptococcus bovis*, *S. equinus*, *Lactobacillus salivarius*, and *L. mucosae* [14–16].

2.2. Simple Sugars Fermenters

Fibrolytic and amylolytic species hydrolyze carbohydrate into simple sugars (cellobiose, glucose, xylose) which are further fermented. The resulting end products of fermentation are SCFAs (most commonly known as volatile fatty acids [VFAs] in herbivores), lactate, and gases (CO_2 et CH_4) (Fig. 1).

The major end product measured after the fermentation of cellobiose by the four cecal strains of *Ruminococcus* sp. isolated by Julliand et al [17] was acetate, whereas little to no lactate was found. On the contrary, amylolytic bacteria are also called lactic acid producing bacteria as they produce lactate as main end product from starch or WSCs fermentation [15,18,19]. *Streptococcus bovis* and *S. equinus* are only L-lactate producers, whereas other strains were reported to produce both isomers of lactate [16]. Lactate is a substrate for lactate-utilizing bacteria, which are able to convert it into VFAs, mainly propionate. The major lactate-utilizing bacteria belong to the Veillonellaceae families and to the *Megasphaera* and *Veillonella* genera. *Peptostreptococcus elsdenii* (now *Megasphaera elsdenii*) and *Veillonella gazogenes* (now *Veillonella alcalescens*) were identified early on as main lactate-utilizing bacteria species in the hindgut [18,20].

The global VFA composition within the hindgut content is determined by the microbiota composition and by the nature of plant carbohydrates in the digesta. The activity of fibrolytic microorganisms induces high levels of acetate (C2) and to a lesser extent of butyrate (C4), whereas the activity of amylolytic microorganisms induces a large increase in propionate (C3) proportion. Thus, the fibrolytic activity resulting from plant cell wall fermentation can be evaluated in horses through the ratio [(acetate + butyrate)/ propionate] ([{C2 + C4}/C3]), as it was suggested in
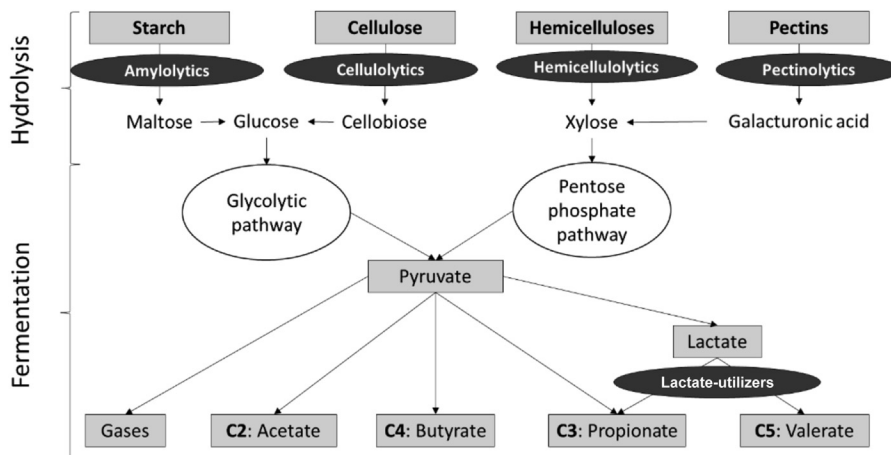

Fig. 1. Pathways of plant complex polysaccharides hydrolysis and fermentation by the hindgut microbiota and resulting end products.

ruminants [21]. The higher the fibrolytic activity is favored over the amylolytic activity; the greater is C2 concentration and thus the higher ratio. In the literature, this ratio ranged between 4.3 and 6.0 in the hindgut of horses fed diets containing 100% forage [22–24].

3. The Hindgut Microbiome and Dietary Changes

In horses, the impact of the diet on the hindgut microbiome has long been demonstrated using culture-dependent techniques [14,23,25–30] and was recently confirmed using culture-independent techniques [31–33].

In the literature, the alterations of the hindgut microbial ecosystem have been assessed under different conditions: most studies examined the impact of abrupt changes and changes between high-starch versus high-fiber diets, while the effect of feed form and processing or feeding frequency was rarely explored.

3.1. Impact of an Abrupt Change of Diets

3.1.1. Abrupt Change Between Two Different Forage Diets

To date, studies assessing the impact of an abrupt change of forage on fibrolytic (cellulolytic, xylanolytic, and pectinolytic) bacterial populations of the horse hindgut microbiota reported no alteration on a short or longer term following the change.

On a short term, cellulolytics, xylanolytics, and pectinolytics were not affected in the cecum and right ventral colon, within the 28 hours following a change between two hays of close botanical and chemical composition [34]. This confirmed previous data reporting no alteration of cellulolytics in the right ventral colon 28 hours after a change from hay to haylage or silage from the same botanical origin [35] and 24 hours after a change between two silages with different crude protein (CP) content [36].

On a longer term, cellulolytics, xylanolytics, and pectinolytics were not altered, either in the cecum or in the right ventral colon, 8 and 15 days after the change between two hays of close botanical and chemical composition [34]. Similarly, cellulolytic bacteria were unchanged in the right ventral colon throughout the weekly observations after a change from hay to haylage or silage from the same botanical origin [35] or between the two different CP content silages [36].

Conversely, some bacterial populations not involved in the breakdown of fiber were modified in the colon of horses undergoing an abrupt change between two different CP silages on a short or longer term. Four hours following the change, the concentrations of total anaerobic bacteria, streptococci, lactate-utilizing and proteolytic bacteria decreased [36]. Also, within the subsequent 3 weeks following the change, total anaerobes, lactobacilli, streptococci, and lactate utilizers counts were modified [36].

In all three studies, botanical origin and/or biochemical composition of the forages were identical or very close, which potentially explained the minimal impact on the hindgut microbiota. Also, the assessment of the bacterial populations was based on cultural techniques in all three studies. The use of rDNA 16S sequencing might bring new

insights about refined changes of the bacterial richness, diversity, and composition.

3.1.2. Abrupt Change Between Forage and Concentrate-Based Diets

Using culture-dependent techniques, the effect of an abrupt incorporation of a novel concentrate in the ration on the hindgut microbiota was studied as early as 1988. It was reported that 24 hours after an abrupt change from 100% chopped alfalfa hay to a concentrate diet composed of 86.7% ground corn and 13.3% soybean meal, the percentage of amylolytic bacteria increased [25]. In the early 2000s, the cecal and right ventral colonic microbiota was shown to be modified as early as 5 hours after an abrupt incorporation of barley in a fiber-based diet. A marked increase of the lactobacilli and streptococci concentrations was found, as well as an increase in the lactate concentration [27,37]. As expected, the colonic lactate-utilizing bacteria concentrations increased 5 hours after the barley incorporation [37]. Although alterations in cellulolytic bacterial concentrations were not significant within 29 hours after a change in diet in the cecum and right ventral colon [27], percentages of xylanolytic and pectinolytic bacteria were reported to decrease markedly in the first days after the change (exact day not specified) [25]. The increase of amylolytic activity and the decrease of fibrolytic activity were consistent with the decrease of the ratio ([C2 + C4]/C3) measured in the proximal hindgut [27].

De Fombelle et al [27] emphasized that the counts and activities of microorganisms showed greater variations in the right ventral colon than in the cecum. They suggested that the first segment of the colon appeared to be the main site affected by overfeeding barley, which might explain why it is a major site affected during colic.

Forty-eight hours after the abrupt change from hay to a concentrate diet, Goodson et al [25] reported a large increase of the total anaerobic bacteria counts (decreasing again by 72 hours), a marked decrease in protozoan numbers (staying at lower values with the concentrate diet), and a decrease in amylolytic counts. De Fombelle et al [27] observed that the changes in microbial counts within 24 hours were temporary and that at 7 days, there were no longer differences between the diets.

In all studies, bacterial populations were studied using culture-dependent techniques. Using culture-independent sequencing would probably bring new information regarding bacterial richness, diversity, and composition changes.

3.2. Alterations Between High-Fiber Diets and High-Starch or High-Soluble Carbohydrate Diets

Recent work using high-throughput next-generation sequencing found a lower richness and diversity of cecal and right ventral colonic bacteria with a concentrate-based diet compared with a hay-based diet [32]. Species-rich and diverse communities are less susceptible to imbalance as different species can have similar functions thus conferring resilience in the hindgut microbial ecosystems. By decreasing bacterial richness and diversity,

starch potentially causes a concomitant decrease in resilience and as a result a higher susceptibility to dysbiosis.

Globally, total anaerobic bacterial concentrations increased in the hindgut when high-starch diet was fed. An increase of amylolytic bacteria [24,25] and associated genera *Lactobacillus* and *Streptococcus*, often larger in the right ventral colon than in the cecum [12,23,24,27,28,37], was also reported. The growth of these lactate-producing bacteria was associated with a marked increase in lactate concentration [23,27,28] for both D- and L-isomers [24]. Indeed, an overgrowth of lactate-utilizing bacteria was associated with the increase of lactate concentration [23–25,28,37] and Veillonellaceae family [32]. Despite the increase of lactate utilizers, a decrease in the proximal hindgut pH was measured with high-starch diets compared with high-fiber diets [25,28,32,38] and a greater decrease was reported in the right ventral colon than in the cecum [24]. In turn, fibrolytic bacteria, which are acid intolerant, declined. Several authors reported a decrease in the concentrations of cellulolytic [23,28,29], xylanolytic, and pectinolytic bacteria [25]. Recent studies using culture-independent techniques confirm that the Lachnospiraceae and Ruminococcaceae families and the *Fibrobacter* spp. have lower abundance in the hindgut of horses fed a high-starch diet compared to those fed high-fiber diet [31,32].

The different alterations of the microbiota tended toward lower fibrolytic activity and higher amylolytic activity of the ecosystem with higher soluble carbohydrate intake. This change was reflected by the decrease of the ratio ([C2 + C4]/C3) which values ranged from 3.1 to 4.3 with a high-starch diet, compared to from 4.3 to 6.0 with a high-fiber diet [23,24,28,38]. Only de Fombelle et al (2003) did not report a change of this ratio, maybe due to the fact that horses were not their own control in the experimental design.

Interestingly, the standard errors for bacterial concentrations in the cecum and colon were greater with a high-starch diet compared to those observed with a high-fiber diet [23,28]. This suggested an individual susceptibility to high-starch diets and demonstrated that some horses are more likely to develop digestive dysbiosis and potential intestinal diseases, such as colic.

3.3. Impact of Cereal Form and Processing

Few results are available in the literature regarding the impact of cereal form and processing on the hindgut microbiota. Philippeau et al [30] fed horses with hay and barley under four different forms: whole grain, 2.5-mm ground, steam flaked, or pelleted. Using cultural techniques, they detected no difference for total anaerobic, cellulolytic, amylolytic, and lactic acid–utilizing bacteria in the colonic content. However, pH, cellulolytic bacteria counts, and the ([C2 + C4]/C3) ratio were numerical numerically lower in horses receiving ground barley. This suggested that thermomechanical (pelleting or steam flaking) rather than mechanical (grinding) processes may limit potential negative impact of starch on fibrolytic activity in the right ventral colon of horses fed a high level of starch. Further research using high-throughput next-generation sequencing of DNA would probably shed light on the main bacterial populations altered under a dietary change in cereal form. Moreover, studying enzymatic activities would be highly valuable to bring complementary valuable information on the microbial activity in the hindgut.

3.4. Impact of Feeding Frequency of Pelleted Concentrate Ration

The effects of meal size and frequency on cecal microbiota have been reported recently in one study using 16S rRNA gene-based sequencing [33]. The authors fed the same, pelleted concentrate ration either in a single or in two or three equal meals each day. They measured bacterial structure and diversity every 2 hours in the cecum. Whereas the bacterial species richness was not different, the microbial abundance was different in horses fed one large meal compared with those fed three smaller meals throughout the day. Alterations were seen at the genus level (YRC22, *Lactobacillus*, *Streptococcus*, *Prevotella*, *Coprococcus*, and *Phascolarctobacterium*). Horses were supplied a maximum of 9 g/kg body weight starch per meal and 6 g/kg body weight WSC per meal. This did not exceed 2 g/kg body weight starch per day that was reported to be at risk for higher quantity of starch to reach the hindgut fermenters [39]. Moreover, it was combined with 6 hours access to pasture and 3 kg of hay.

4. Representativeness of the Fecal Microbiome Under Diet Changes

Following from the hindgut ecosystem, feces also harbors a dense microbiome [40,41]. Feces is already used by many researchers to study the hindgut microbial ecosystem and its variations under dietary change, as fecal matter is easier to collect than hindgut content, and facilitates monitoring horses over a long term. As discussed in Julliand and Grimm's recent review [13], feces is not representative of the proximal part of the hindgut: its microbial structure differs from that found in the cecum and ventral colon, which are the main compartments for fibrolysis. However, the fecal microbiota seems also sensitive to dietary variations (Fig. 2).

4.1. Modification of the Fecal Bacterial Diversity and Composition

Reduced fecal diversity was observed in the feces of horses fed high-starch diet compared to horses fed high-fiber diet [42]. Moreover, the fecal diversity increased rapidly within 4 days following an abrupt transition from an ensiled conserved forage-grain diet to pasture [40], suggesting that change in the fecal microbiome can occur in a short time after dietary change.

When comparing bacterial abundance in feces of high-fiber or high-starch fed horses, Proteobacteria increased with a high-starch diet [42]. Among this phylum, the genera *Succinivibrio* increased in high-starch fed horses [42]. Oppositely, the order Clostridiales and the Lachnospiraceae family decreased [42] as did a number of unclassified members within the order Clostridiales and
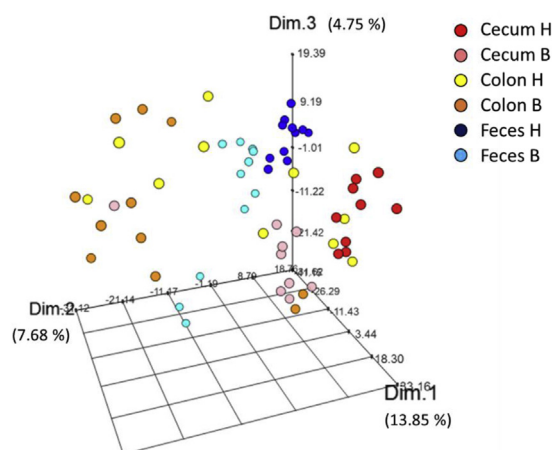
Fig. 2. Principal component analysis showing the bacterial structure in the cecum, right ventral colon, and feces of horses fed either 100% DM hay (H) or 56% DM hay + 44% DM barley (B) using sequencing techniques (Grimm et al, personal data). DM, dry matter.

within the family Lachnospiraceae [40]. Together with the Ruminococcaceae, Lachnospiraceae were identified as important fibrolytic bacteria [43]. These two families, which decreased in the hindgut of horses consuming concentrate diet [31,32], were reported to be key components of the mammalian gut health [43]. These families could have the potential to degrade cellulose. Whereas fecal cellulolytic concentrations measured using culture techniques showed no variations in horses fed either high-fiber or high-concentrate diet in three studies [29,44,45], it was reported recently that the number of cellulolytic bacteria decreased in horses fed high-starch diet compared to a hay control diet [46].

Using cultural techniques, an increase of the amylolytic bacteria (streptococci and lactobacilli) count in feces was observed in studies testing a high-starch diet versus a high-fiber diet [46–48], as was an increase of the lactate-utilizing bacteria [44,49]. Changes in streptococci and lactobacilli relative abundance were, however, not reported by studies using molecular-based techniques [40,42].

All these findings corroborated the responsiveness to the diet of the fecal microbiome. However, the majority of studies did not verify if the variations observed in feces represented variations of the hindgut microbiome.

4.2. Variations in Feces in Relation With Variations in the Proximal Hindgut

Few studies confirm if similar patterns of variation occur both in the feces and in the hindgut. Until recently, only two studies were published which were specifically designed to measure the impact of a high-starch diet versus a fiber-rich feed on the bacterial profiles of the hindgut and the feces using culture-dependent techniques [29,44]. de Fombelle et al [29] reported no variations in the fecal bacterial concentration when the diet was changed, although the cecal and right ventral colonic bacterial concentrations were altered. Conversely, Julliand and Goachet

[44] showed that the fecal microbiota could be an appropriate indicator of bacterial changes in the right ventral colon.

Very recently, a study transitioned horses from high-fiber to high-starch diet and observed that the functional bacterial group concentrations (cellulolytics, amylolytics, and lactate utilizers) were significantly correlated between cecum and feces and between right ventral colon and feces [24]. This demonstrated that fecal samples and their bacterial analyses could be used to represent proximal hindgut microbiome in terms of variations during a change from a high-fiber to a high-starch diet and thus could be markers of particular interest to follow proximal hindgut microbial dietary-related variations.

5. Conclusions

In horses fed a hay-based diet, the bacterial community shows a high richness and diversity suggesting a strong resilience in the hindgut microbial ecosystems. However, athletic horses are submitted to different feeding practices such as large meals of concentrate-based diet or abrupt changes of feeds. Studies indicate a clear effect of high-starch diet on microbial populations in the hindgut. This highlights the importance of managing the concentrate feeding to the horse for health and welfare. There is a need for further investigations regarding other dietary practices on the hindgut microbiome in order to limit their potential detrimental impact. Also, better knowledge of the hindgut microbiota in horses maintained on pasture is required. Up to date, one study reported data on that topic using fecal collection [41].

The gold standard for conducting experimental trial assessing the impact of the diet on the hindgut microbiota is the use of fistulated animals. This allows the direct access to the hindgut content and limits variations between individuals when repeated measures are done. However, future investigations might be facilitated by the use of feces which appears to be good indicator of hindgut microbiota variation under dietary changes.

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