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The Impact of High-fiber Diet and High-starch Diet on Equine Gut Microbiota

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Abstract:

To better understand the health of horses, analyzing the equine gut microbiota is an inevitable step. Feeding strategies, as one of the main factor that significantly affects the microbiota composition of equine gut, has gradually become the focus of research. This paper systematically analyzes the different effects of two different dietary structures, high fiber and high starch diets, on the equine intestinal microbiota. The results of this paper show that high fiber diets can maintain a high level of alpha diversity in the equine hindgut microbiota and promote the growth of beneficial bacteria. However, high starch diets decrease microbiota α-diversity, increase the number of potentially pathogenic bacteria, and are associated with diseases such as colitis and acidosis. In addition, this paper presents recommendations for proper feeding of horses based on the latest research, highlights the importance of high-fiber diets by comparing the two diets, and summarizes some feeding recommendations to provide horse breeders with the right feeding ideas, aiming to contribute to the field of equine health.

Keywords: Equine gut microbiota; high-fiber diet; high-starch diet; equine dietary modulation.

1. Introduction

The gastrointestinal microbiota in horses, like humans, plays a central role in digestion, immune function and performance. However, unlike humans, horses rely on microbial fermentation to produce short chain fatty acids (SCFAs) to provide more than half of their energy needs [1]. Microbial fermentation primarily occurs in the cecum and colon, which makes the composition of their gut microbiota an important entry point for understanding their health and performance. The gut microbiota of horses is highly sensitive, and various factors have multiple impacts on it. Some disturbances caused by external or internal stressors can lead to digestive system diseases in horses, such as gastric ulcers, colic, diarrhea or colitis, which are believed to be related to systemic diseases such as laminitis, equine metabolic syndrome, or obesity.

However, with the domestication of horses, their timing and composition of feed were changed. are not allowed to move freely and feed freely on the grassland. They are confined in boxes, restricted in their movements and feed at fixed times. For performance horses, breeders always use energy dense feedstuffs

to replace forage for energy supply [2]. These modern breeding modes will disrupt the equine gut microbiota and cause to health issues. Therefore, diet, as one of the direct factors that will affect the equine gut microbiome, should be valued by people.

Currently, the most popular and widely used techniques are high-throughput DNA sequencing methods, especially next-generation sequencing (NGS) because of their cost-effectiveness, high resolution and scalability. NGS encompasses two main strategies: marker gene sequencing and shotgun metagenomic sequencing. And between these two strategies, marker gene sequencing targets specific genetic markers. For instance, the 16S rRNA gene is targeted for bacteria, the 18S rRNA gene for eukaryotes, and the internal transcribed spacer (ITS) region for fungi [3].

With the help of NGS, studies of equine gut microbiome have made significant progress. Lots of international studies indicate that HF diet is able to maintain high microbiota alpha diversity in the hindgut of horses and increase the abundance of Fibrobacteraceae which can increase the production of SCFAs and *Prevotella* which is linked to better performance in horses. Conversely, microbiota α-diversity is reduced under HS diet, increases the relative abundance of potentially pathogenic bacteria like *Streptococcus* and *Fusobacterium* and increases the abundance of Succinivibrionaceae which is related to acidosis. However, in China, research on the intestinal microbiome mainly focuses on humans, rare animals and husbandry. Thus, studies on the intestinal microbiome of horses are relatively scarce. Although some teams in China have begun to pay attention to how the equine intestinal microbiota influence metabolism and immunity of the host, the overall research still lags behind the international advanced level.

As HS diet is often used in performance horses and its risk of gastrointestinal diseases, it is necessary to systematically integrate the research results on how HF and HS diet affect the gut microbiota. This article aims to clarify the influence of these two dietary regimes on the intestinal microbiota ecosystem of horses through a comprehensive review. Through this research integration, I expect to reduce the situation where breeders feed their horses a high-starch diet due to incorrect perceptions and lay the foundation for the development of precise nutrition strategies.

2. Fundamental Characteristics of Equine Gut

2.1 The Community Structure of Equine Gut

The gut of horses is complex and contains various microbial communities, including viruses, archaea, bacteria, protozoa, fungi and parasites [3]. These microbial populations can affect the physiology, metabolism, nutrition and immune functions of the horses [4]. Among them, bacteria were numerically dominant because they play crucial roles in digestion, metabolism and interaction with the host. Firmicutes and Bacteroidetes dominate the microbial composition at the phylum level. Then, to a lesser extent, include Fibrobacteres and Spirochaetes. The phyla found at the lowest relative abundances are Verrucomicrobia and Proteobacteria. In the phylum of Firmicutes, Clostridiales, Ruminococcaceae and Lachnospiraceae families are particularly found across the hindgut of horses, and are mainly responsible for the fermentation of plant fibers as well as the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. These acids provide most of the energy of the horses' daily energy needs [3]. Bacteroidetes such as Prevotellaceae and Paraprevotellaceae also play important functions in fiber catabolism and polysaccharide utilization. Furthermore, the gut microbiome can regulate host intestinal and systemic immunity as well through microbial metabolite signaling pathways. Overall, the microbiome in the equine gut not only plays an important role in its digestive metabolism but is also an entry point for scientists to understand its health and potential disease risks.

2.2 Spatial Heterogeneity of Equine Gut

The horse is a typical example of a ruminant herbivore, unlike other ruminants, it relies more on gut microbiota to break down fiber. The food consumed by horses quickly enters the cecum and colon through the stomach and small intestine (about 2-3 hours) for fermentation. Thus, the ecological microbial community structure in hindgut is much more complex. In the foregut which includes the stomach and small intestine, the microbiota composition is dominated by Firmicutes and Proteobacteria, such as Lactobacillaceae and Streptococcaceae. These microorganisms play essential roles in the fermentation of soluble carbohydrates, proteins and starches [1]. In the hindgut which includes the cecum and colon, the physiological conditions differ from the conditions in the foregut. The time taken for transit is longer, and the pH value is lower compared with a small intestine and an anaerobic environment that benefits the fermentation of recalcitrant fibers. As mentioned before, Firmicutes (Clostridiales, Lachnospiraceae, Ruminococcaceae) and Bacteroidetes (Prevotellaceae, Paraprevotellaceae) dominate the hindgut. Compared with the foregut, the hindgut contains less Proteobacteria and lactic acid bacteria. This kind of heterogeneity of microbiota composition makes horses more sensitive to external stimuli, for instance, diet structure and environmental stress. Understanding the spatial het-

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erogeneity of the equine gut allows scientists and farmers to prevent and interpret the occurrence of dysbiosis and gastrointestinal disease, as well as optimize dietary strategies for horses.

3. Host-Associated Influencing Factors

The microbiota composition of the equine gut is affected by a range of host-related factors, including age, diet, genetics, health status and domestication status, etc. Age as one of the biggest factors that affect the microbiota composition of horses, reflects the dietary adaptation of horses. Foals' gut microbiota is mainly composed of simple fermentative microorganisms, such as Bacteroides, which aim to a function of breaking down lactose and milk protein as foals rely on breast milk. In contrast, the adult equine gut microbiota is dominated by complex carbohydrate-degrading bacteria which reflects its adaptation to a high-fiber diet [5]. The genotype of the host affects the intestinal microbiota population of horses congenitally. For example, the abundance of Proteobacteria, Synergistetes, Planctomycetes, Chloroflexi and TM7 shows a huge difference between Mongolian and Thoroughbred horses. *Treponema* identified in Mongolian horses, at the genus level, showed a considerably greater abundance, where Mongolian horses contain 43% of *Treponema* while Thoroughbred horses contain only 29% of it [4]. Health status profoundly shapes the equine gut microbiota composition. The use of antibiotics will decrease the diversity and increase the opportunity for pathogenic taxa colonization. Multiple studies have shown that horses with diarrhea/colitis generally have the characteristics of reduced microbial diversity and dysbiosis. However, there were also conflicts among different studies. Some studies reported the abundance of *Lactobacillus* increased while some reported the abundance of *Lactobacillus* decreased [6]. But the fact that cannot be ignored is the crucial role of microbial balance in equine intestinal health. The domestication status of horses affects the microbiota composition as well. Based on the experimental results, domestic horses have higher archaea levels and lower levels of eukaryotes and viruses compared with wild horses. In general, domestic horses have lower diversity in microbiota composition compared with wild horses [7].

4. The Regulatory Mechanism of Feed Type and Structure on Equine Gut Microbiota

Diet is one of the biggest and most important factors that influence the equine gut microbiota. The different types of feed, especially the difference between starch and fiber content, influence the hindgut microbiota composition and function notably. Feed can be divided into three different types with different functions. Hay, silage, fresh forage and fiber-rich byproducts are considered Forage, which is the foundation of a horse's diet and mainly provide fibers like Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) to maintain intestinal health[2]. Fat-rich feeds and Cereals grains like corn, wheat and oats are considered as Concentrates, which is then used to support exercise and growth of Racehorses by providing high energy. In general, feed structure can be divided into two different types: high-fiber (HF) diet and high-starch (HS) diet. High-fiber diet is always related to the high proportion of forage and HS diet is always related to a high proportion of concentrate.

The characteristic of a high-fiber diet (HF) is taking a rich of forage. It has been proved that it is able to promote a healthier hindgut microbiota. In a controlled 129-day feeding trial, horses on HF vs. high-starch (HS) diets exhibited significantly higher microbiota alpha diversity [8]. Additionally, in the hindgut of HF diet-fed horse, there is a greater abundance of Fibrobacteraceae (3.8% under HF diet versus 0.3% under HS diet in caecum and 4.3% under HF diet versus 0.5% under HS diet in sternal flexure) and *Prevotella* (16.5% under HF diet compared to 6.9% under HS diet in the sternal flexure). Fibrobacteraceae can maintain the energy-harvesting efficiency of horses and raise the production of SCFAs. Meanwhile, *Prevotella* is linked to higher performance in horses [9]. These results fully reveal the benefits of HF diet for horses.

In contrast, a long period of consuming a high starch diet led to a significant decline in the richness and evenness of the gut microbiota, as the caecum, pelvic flexure, and right dorsal colon all exhibited a decrease in microbiota alpha diversity [8]. The decrease in diversity not only reflects the decline of gut microbiota stability but also indicates that the intestinal ecosystem will have a weaker ability to resist external disturbances.

The changes in microbiota caused by HS diet are particularly obvious at genus or family taxonomic ranks. In the small intestine that includes the jejunum and duodenum, HS diet led to a significant increase in the relative frequency of Enterobacteriaceae (0.7% under HF diet versus 7.5% under HS diet in jejunum and 0.7% under HF diet versus 1.1% under HS diet in ileum) [8]. The Enterobacteriaceae family contains a variety of potential pathogenic bacteria: *Escherichia coli* and *Salmonella* are two examples. The overgrowth of them will not only cause dysbiosis which disrupts the original microbial balance but is also directly linked with colitis [3]. In the hindgut, HS diet lowers the relative abundance of Prevotellaceae and Fibrobacteraceae respectively in the caecum and sternal flexure. These two

microbial groups both play vital roles in the fermentation of plant structural carbohydrates. Decreasing their relative abundance may weaken the degradation capacity of plant cell wall polysaccharides and further cause the reduced energy harvesting efficiency of the host from crude fiber substrates. More importantly, HS diet also promotes the proliferation of pathogenic bacteria. The research found greater levels of *Streptococcus* (1.9% under HS diet compared to 0.4% under HF diet in the cecum) and *Fusobacterium* that only found in HS diet horses (0.04% under HS diet compared to 0% under HF diet in sternal flexure) separately, where both of them are pathogenic bacteria that give rise to inflammation diseases. In addition, the abundance of Succinivibrionaceae in pelvic curvature and rectal regions is also increased due to HS diet. Members of this bacterial family can ferment starch into short-chain fatty acids quickly and produce gases like hydrogen and carbon dioxide [10]. Under high starch conditions, their metabolic activity may lead to the accumulation of acidic fermentation products and a drop in pH value, which makes this bacterium a type of amylolytic bacteria associated with acidosis [8].

Finally, in the rectal microbiota, horses fed with HS diet exhibited a significantly lower relative abundance of Lachnospiraceae and Ruminococcaceae compared to those on an HF diet [8]. A reduction in their abundance may compromise mucosal health, impair epithelial energy metabolism, and increase the risk of endotoxin translocation. While HF diet enriched these 2 bacteria, which better support epithelial barrier, anti-inflammatory ability and energy metabolism. Collectively, these findings highlight the negative consequences induced by HS diet and the importance of HF diets as the foundation of equine nutrition to prevent microbial dysbiosis.

5. Suggestions for Proper Equine Feeding Protocol

Based on the existing studies about the impact of HF and HS feeds on the equine gut microbiota, here are some suggestions for feeding horses. The consequence of consuming HS diet for a long time shown above indicates that the proportion of starch, which is mostly in the concentrate, should be decreased in horses' daily diet. As HF diet can raise promote a healthier hindgut microbiota by raising the abundance of Fibrobacteres and Bacteroidetes and maintaining a high microbiota alpha diversity, forage that is longer than 2.5cm should be the must-needed feed in horses' daily diet. 15 grams of dry matter should be consumed per kilogram of body weight every day (15 g DM/kg BW) for a normal horse [2]. To guarantee horses' welfare, forage should be fed to horses ad libitum or spread throughout the day to avoid the fasting states of horses that are longer than 4 hours [2, 11].

In addition to these basic feeding principles, the following considerations should be taken into account when feeding horses with forage as well. In terms of forage quality, Breeders must regularly check the hygiene of forage both visually and olfactorily. Moldy or deteriorated feed should be discarded instead of continuing feeding to the horses [2]. Microbial contamination, such as mold and bacteria, can cause respiratory diseases, digestive problems or even death in horses. In terms of changing feed type, a transitional adaptation period of 2 to 3 weeks should be set to ensure the sensitive equine gut has enough time to adapt to a new type of forage that has different nutritional components compared with the previous one. Sudden changes in feed may lead to dysbiosis.

Based on the previous conditions, concentrate can be fed to horses combined as an auxiliary energy source. It is worth noting that the feeding of concentrate should prevent high-dose concentrated feeding in a single meal. For horses that require high energy consumption, concentrate can be divided into smaller amounts each time and fed to the horses in frequent meals. Therefore, to reduce the accumulation of starch.

To maximize the welfare of horses, breeders should adjust their dietary strategies under the guidance of veterinarians according to the different conditions of each horse. To customize the diet for each horse, nutritional analysis on different forages is recommended to precisely adjust them based on the actual situation, especially for those horses with diseases [2]. In addition, probiotics and prebiotics (such as mannose oligosaccharides) can also be added to the diet of horses to improve their digestion, metabolism, growth and immunity. Meanwhile, reduces the stress and incidence of intestinal diseases [12]. However, current reports about pre-/probiotic are still relatively few, and the research methodology is not yet mature enough (e.g. too less horses enrolled per group, performed in vitro, included lots of variables, etc. [6]).

6. Conclusion

This paper critically discussed the impacts of HF diet and HS diet on equine gut microbiota. Through comparing and contrasting the existing studies, HF diet can bring more benefits to equine gut health than HS diet. The benefits brought by HF diet include maintaining a high microbiota alpha diversity in the hindgut, enriched Lachnospiraceae and Ruminococcaceae to produce SCFAs to support the energy metabolism, together with increasing the abundance of beneficial bacteria that are related to higher production of SCFAs and higher performance such

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as Fibrobacteraceae and *Prevotella*. In contrast, HS diet brought several negative impacts on the equine gut health, including a decrease in lower microbiota alpha diversity, decreased gastrointestinal function, increases in the abundance of Succinivibrionaceae which can cause acidosis, and an increase in the relative frequency of Enterobacteriaceae which encompass potential pathogenic bacteria linked with dysbiosis and colitis. After comparing and analyzing the diets of HF and HS, recommendations for appropriate feeding plans were summarized based on existing research, including basic feeding principles, the importance of feed quality, how to adapt horses to new feed, and if possible, customized diets for horses. However, current research still has limitations. There is insufficient research on the use of probiotics and prebiotics in horses, which also applies to methods for detecting the gut microbiota of horses, such as in vitro fermentation models and fecal samples. This reflects that there are still many areas in the field of horse gut health that need to be investigated and explored. Future research should focus on studying the digestive physiology of horses, combining longitudinal microbiome analysis with host performance, and developing new fermentation systems. Therefore, exploring personalized and precise feeding plans, and researching new probiotic products. By understanding how diet regulates the gut microbiota of horses, precise nutritional strategies can be developed to improve their welfare, which can support their future health and performance.

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