



Research Progress on Influencing Factors of Gastrointestinal Microbial Diversity in Equine

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ABSTRACT

Microbiota in the gastrointestinal tract play a crucial role in nutrient digestion, health and so forth in equines. As the research attention on gut microbes has increased, several studies have investigated the composition of the gastrointestinal microbial flora in equines. This article reviews the effects of breed, age, intestinal site, nutritional management and diseases on the gastrointestinal microbiota of horses and donkeys, thus offering references for improving the gastrointestinal microecological environment in these animals and preventing and controlling disease occurrence in them.

1 | Background

Equines, including horses and donkeys, are monogastric herbivores with unique anatomical structures and diets. They exhibit hindgut fermentation and have a well-developed hindgut structure, especially cecum.

Microflora refers to a group of microorganisms that live in or on a host and interact with it in a unique manner, and the microflora composition can vary considerably because of environmental factors and host health. Nearly 80% of gut microbes cannot be isolated using conventional methods (Langner and Vervuert 2019). Some studies on the microbial composition of the digestive tract of healthy horses have reported that microbial distribution varies in different parts of the gut (Costa et al. 2015). Among

them, bacterial phyla such as *Firmicutes*, *Bacteroidetes* and *Verrucomicrobia* dominate the hind intestines of horses. These microbes work together to help horses break down complex plant polysaccharides, such as cellulose, to produce important sources of energy, such as short-chain fatty acids (Wunderlich et al. 2023). In addition, certain microorganisms, such as methane-producing archaea, produce methane by metabolizing hydrogen and carbon dioxide, supporting the activity of cellulose-degrading bacteria (Kauter et al. 2019). This variation results from the differences in the oxygen level, acid–base level and temperature of different parts, which leads to the presence of distinct microbial strains in each part. Of these parts, the large intestine has the highest number of bacteria. The caecum seems to be a third separate ecosystem with numerous IgA+ cells and a diverse microbiota (Żak-Bochenek et al. 2024).

Wei Zhang and Rong Guo have contributed equally to this work.

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The gut microbiota constitute a complex ecosystem with varied functions. In studies investigating gut microbiota in donkeys, a complex relationship was observed between the gut microbiome and expression of genes, particularly those involved in the immune system (Li et al. 2022). In addition, several studies have demonstrated that equine faecal microorganisms are closely associated with factors, such as digestion (Juśkiewicz et al. 2021; Zaitseva et al. 2023), metabolism (Zhu et al. 2021), obesity (Walshe et al. 2021) and disease (Lara et al. 2022). This article mainly reviews the effects of breed, age, intestinal site, nutritional management and diseases on the gastrointestinal microbiota of horses and donkeys, thereby offering a reference for improving the gastrointestinal microecological environment in these animals and preventing and controlling disease occurrence in them.

2 | Effects of Breeds on Gastrointestinal Microbes

Microorganisms in the gastrointestinal tract have a mutually beneficial symbiotic relationship with their hosts, and the microbial composition varies in different species. *Firmicutes* and *Bacteroidetes* were found to be the most abundant and predominant phyla in Mongolian horses and thoroughbreds, and 30 of the 75 phyla differed significantly between the 2 horse breeds, such as *Synergistetes*, *Planctomycetes*, *Proteobacteria*, *TM7* and *Chloroflexi*. Compared with thoroughbred horses, Mongolian horses had higher levels of *Treponema*, whereas lower levels of *Oscillibacter*, *Fibrobacter*, *Methanocorpusculum* and *Succinivibrio* (Zhao et al. 2016). While assessing the faecal microbiomes of feral and domestic horses, Ang et al. (2022) found that the faecal microbiota of domestic horses were impoverished, possibly because of their diet, antibiotic exposure and hygiene.

Liu et al. (2020) reported that wild donkeys had higher dry matter digestibility and a relatively complicated bacterial network in the gut than domestic donkeys, such as higher levels of *Bacteroidetes*, *Firmicutes* and *Ruminococcaceae_NK4A214*. Furthermore, 29 of the 40 metabolic pathways, mainly including amino acid, carbohydrate and energy metabolism, were present in the wild donkeys. Additionally, wild donkeys from the Tibetan Plateau exhibited gut microbiota different from those of donkeys from other regions, which helped them to adapt to the high altitudes (Liu et al. 2020). By contrast, domesticated animals usually live in artificially controlled environments and have relatively simple microbiota, possibly because of factors such as dietary habits and antibiotic use (Table 1).

3 | Effect of Age on Gastrointestinal Microbes

Numerous experiments have confirmed that differences in the intestinal flora of horses are related to their age. Newborn horses have a complex gut microbiota. At birth, microorganisms colonize the gastrointestinal tract of newborn foals through the breast milk they feed upon and other routes, and the microbial community of these foals is thus reorganized (Costa et al. 2016). Digestive microbiota of newborn horses continuously adapt to changes in dietary requirements and food types, that is, changes in the composition of mare milk or the transition from milk to feed diet (De La et al. 2019). On conducting second-generation sequencing of meconium from 13 pairs of standard-bred foals, Quercia et al.

(2019) found that microbial components derived from the mare symbiont communities established in the gut of foals since their foetal life. After birth, an external route for the transmission of mare microorganisms is formed. Faubladier et al. (2014) analysed the faecal samples of five foals and revealed that the intestinal bacterial community in foals is established sequentially, and this process reaches its climax state when they are around 1 month of age. Costa et al. (2016) studied 11 mare-foal pair samples by longitudinally collecting faecal samples from mares and foals on the first postpartum day and periodically again until the foals were 9 months of age. They reported that microbiota reached relative stability from foal birth in 60 days. Lindenberg et al. (2019) observed that the gut microbiota stabilized within 50 days of foals after delivery. Older horses (age: 19-28 years) have less diverse gut microbes compared with adult horses (age: 5-12 years) (Dougal et al. 2014). Colonization of the gut by segmented filamentous bacteria in equines may also be related to their age (Lowden and Heath 1995). Gastrointestinal microbiota may also vary because of differences in sampling timing and feeding methods, as well as because of the effects of delivery mode and breastfeeding on development and function of these microbiota (Bäckhed et al. 2015).

Functional prediction of faecal samples of donkeys of different ages revealed that the diversity and richness of the gut microbiota of foal donkeys (≤3 months) were significantly different from those of adult donkeys (≥7 months) and that this diversity increased with age. However, the microbial composition changed significantly in the first month (Xing et al. 2020). The number of Bacteroidetes, Lactobacillus and Odoribacter decreased with age, whereas that of Streptococcus increased significantly. Regarding functional prediction using faecal samples, significant differences were observed in the relative abundance of pathways across age groups. The microbial community composition and structure are uniquely characterized in each age group, which is consistent with the functional stability of these communities. Microbial diversity in faeces increased with the age of the donkeys, suggesting that age is a critical factor affecting microbial succession. This implies that breast milk composition may affect initial gut microbiota composition.

In summary, the gut microbiota of equids exhibit significant changes across age, which are reflected both in their microbial composition and functionality (Table 2). The gut microbiota of foals and young animals are particularly dynamic, initially colonizing through external sources such as breast milk and maturing with animal growth and development. The functionality of the gut microbiota also stabilizes over time, which is closely linked to increased digestive and metabolic efficiencies. Understanding these age-related changes can help in providing useful insights into equine animal health and nutrition.

4 | Characteristics and Comparison of Microflora in Intestinal Sites

Equines are herbivorous non-ruminants and have a highly compartmentalized gastrointestinal tract that allows them to digest various plant feed (Harris et al. 2017; Santos et al. 2011). Each compartment of this tract has a semi-isolated ecosystem, and microbiota composition and function of these ecosystems

TABLE 1 Differences in gut microbial composition, dry matter digestibility and metabolic pathways of different breeds of equines.

Microbial composition	Mongolian horses	Thoroughbred horses	Wild donkeys	Domestic donkeys
Firmicutes	High	High	High	High
Bacteroidetes	High	High	Higher	Lower
Treponema	Higher	Lower	Lower	Lower
Oscillibacter	Lower	Higher	Higher	Lower
Fibrobacter	Lower	Higher	Higher	Lower
Ruminococcaceae_NK4A214	Not mentioned	Not mentioned	Higher	Lower
Metabolic pathways	Fewer	Fewer	More complex	Less complex
Dry matter digestibility	Not mentioned	Not mentioned	Higher	Lower

TABLE 2 | Characteristics of microbiological changes in equines of different ages.

Animal species	Age-related changes	Key microbial shifts	Functional implications
Horses (foals)	Complex microbiota at birth, reorganized through breast milk and dietary changes	Transition from mare milk to solid feed influences microbial community	Microbial diversity stabilizes at ~60 days, influenced by feeding and environment
Horses (older)	Decrease in diversity with age (19–28 years)	Reduced microbial diversity compared to adult horses (5–12 years)	Aging leads to less functional diversity in the gut microbiota
Donkeys (foals)	Significant microbial changes in the first month	Decrease in Bacteroidetes, Lactobacillus and Odoribacter; increase in Streptococcus	Increasing microbial diversity with age; suggests functional maturation of microbiota
Donkeys (adults)	Greater diversity and stability in gut microbiota	More stable microbial community composition	More efficient digestion and nutrient absorption as microbiota matures

play a crucial role in animal health and metabolism. According to Costa et al. (2015), the bacterial composition of the equine gut varies widely among compartments, especially at lower taxonomic levels. Although adjacent sites have more similar bacterial compositions, they differ in the mucosal composition and lumen content. On comparing the microbiota at different locations in the gastrointestinal tract of Mongolian horses, Su et al. (2020) found that the microbial community composition differed significantly in the different regions. Significant differences were observed in the microbial community structure of the small and large intestines, and similar patterns were observed in the caecum and colon. Firmicutes (65%) and Proteobacteria (23%) were the most prominent in the foregut, and Firmicutes (45%) and Bacteroidetes (42%) were the most prominent in the hindgut. The caecum is a very important digestive organ in equines. It predominantly consists of proteolytic bacteria, starch-decomposing bacteria, glucose-decomposing bacteria, cellulolytic bacteria and hemicellulose-decomposing bacteria (Dicks et al. 2014). The microbial diversity of the equine colon and caecum is very similar (Reed et al. 2017). Moreover, several studies examining the gut microbiota in different regions of the equine gastrointestinal tract through 16s rRNA sequencing have noted significant differences in the microbiota in the different regions (Chaucheyras-Durand et al. 2022). The faecal microbiota is not informative of the proximal hindgut but can provide insight into the communities of distal compartments (Reed et al. 2021). The caecum has a high number of IgA+ cells and a diverse microbiome (Żak-Bochenek et al. 2024). The uniformity of the gut microbiota was considerably higher in the caecum and colon than in the stomach, jejunum and ileum (Ericsson et al. 2016).

Liu et al. (2019) reported that different parts of the gastrointestinal tract of Dezhou male donkeys had abundant and diverse microorganisms. Moreover, the richness and diversity of hindgut microorganisms were considerably higher than those of the foregut. The gastrointestinal tract microbiota correspond to the physiological functions of the different sites at which they are located. For example, foregut microbes are more active in carbohydrate metabolism, whereas hindgut microbes are more active in amino acid metabolism. The common findings of these studies are that the richness and diversity of the hindgut are higher than those of the foregut, and the foregut and hindgut have different key metabolic aspects.

To sum up, significant regional differences in microbial communities exist within the gastrointestinal tract of equines, and these differences are reflected in the microbial composition and influence the metabolic function of each region (Table 3). The

TABLE 3 | Characteristics of microorganisms from different intestinal sites in equines.

Gastrointestinal region	Dominant microbial phyla	Microbial diversity	Metabolic activity
Foregut	Firmicutes (65%), Proteobacteria (23%)	Relatively lower	Active in carbohydrate metabolism
Hindgut	Firmicutes (45%), Bacteroidetes (42%)	Higher, more diverse	Active in amino acid metabolism
Caecum	Various proteolytic, starch-decomposing, glucose-decomposing, cellulolytic bacteria	Very diverse, similar to colon	High IgA+ cells, crucial for immune and digestive functions
Colon	Similar to caecum	Very diverse, stable	Supports similar metabolic functions as caecum

composition of the microbial communities of the foregut and hindgut differed significantly, with the richness and diversity of the microbial community of the hindgut being generally higher than those of the foregut. Comprehending these regional differences is vital for improving equine health, nutritional levels and metabolic efficiency. In particular, the caecum and colon play key roles in digestion and immune function, and the diversity in their microbial communities is closely related to their functions.

5 | Effects of Nutrition and Management on the Gut Microbiome

5.1 | Fibre and Starch

Diet is a major driver of bacterial diversity among species. Protein- and fat-rich diets increase Firmicutes abundance in the faecal microbiota of humans, whereas dietary fibre increases the abundance of Bacteroidetes and cellulose and xylan degradants (De Filippo et al. 2010; Wu et al. 2011). According to culture-based microbiology, high-starch diets were associated with increased numbers of Lactobacillus equi (currently L. equi) and Streptococcus and decreased numbers of cellulolytic bacteria, compared with high-fibre diets (Medina et al. 2002). Diets with a very high fibre content can affect the microbiota structure in the equine caecum (e.g., the abundance of cellulolytic bacteria significantly increased with a high-fibre diet) (Goodson et al. 1988). Warzecha et al. (2017) observed that the abundance of Paraprevotellaceae, Veillonellaceae and Succinivibrionaceae in the caecum was higher in high-starch diet-fed horses than in low-starch diet-fed horses. In horses fed a starch-rich diet (greater than 1 g/kg body weight per meal), enzymatic amylolysis in the foregut does not lead to the digestion of a large amount of starch (Harris and Dunnett 2018). When the starch reaches the hindgut, it is fermented, and the number of amylase-producing bacteria and lactic acid bacteria increases (Dicks et al. 2014; De Fombelle et al. 2001), thereby producing more lactic acid (Richards et al. 2006). This production of excess lactic acid and volatile fatty acids (VFAs) in the gastrointestinal tract, especially in the stomach, results in a pH drop in the large intestine, which then restricts the proliferation of beneficial and acid-intolerant cellulolytic bacteria (Garber et al. 2020). A large number of bacteria susceptible to excess lactic acid die, and excessive lactic acid deposition can also lead to acidosis (Garner et al. 1977). Thus, the pH of the caecum and colon may decrease with an increase in the number of bacteria that metabolize starch to produce lactic acid, thereby creating adverse conditions for the proliferation of beneficial cellulolytic bacteria. This may interfere with normal digestion. Because of their digestive system, equines can consume small amounts of high-fibre diets for a long period (Elia et al. 2010), whereas high-starch and low-fibre diets may cause hindgut microbial disturbances and intestinal pain in these animals. This dietary stress from high-fibre, low-fibre to high-starch diet significantly increases the abundance of total anaerobic bacteria, lactic acid-utilizing bacteria, and amylolytic bacteria in equines. In a sociability test, the duration of vigilance positively correlated with the concentration of starch-degrading bacteria in the caecum and colon of horses fed a 43%-57% hay barley diet. In the neophobia test, the duration also positively correlated with the number of bacteria utilizing caecal lactate and colonic starch (Destrez et al. 2015). Broom (2006) hypothesized that stress induces immune responses, such as granulocyte proliferation and activation. According to Willing et al. (2009), a fibre-only diet reduced lactobacilli levels and improved microbial stability, thereby preventing associated gastrointestinal diseases. Bergman (1990) reported that total VFAs in the hindgut of whole rough dietfed horses included 75% acetic acid, 15% propionic acid and 10% butyric acid. Furthermore, Bergman reported that the content of acetic acid, propionic acid and butyric acid varied by diet type, and fibre-rich diets increase acetic acid content. Diets rich in unstructured carbohydrates (monosaccharides) or starches increase propionic acid production. These correlational studies have suggested that diet-induced modulation of the microbiota composition influences horse behaviour and induces diseases.

5.2 | Effects of Types of Hay

Sorensen et al. (2021) noted that horses fed alfalfa had greater faecal than caecal pH ($p \le 0.05$), whereas horses fed brome had higher caecal than faecal pH ($p \le 0.05$). Regardless of hay type, VFA concentrations and Bacteroides richness were higher in the caecum than in the rectum. Moreover, alpha diversity was greater in the rectal samples than in the caecal samples (Sorensen et al. 2021). Daly et al. (2012) noted that colonic samples from concentrated diet-fed horses had higher levels of Bacteroidetes than those from pure grass diet-fed horses. Compared with concentrate-fed horses, grass-fed horses had higher numbers of Fibrobacteres and Lactobacillaceae bacteria that degrade fibre, and these microbes may be inhibited by an acidic environment (Daly et al. 2012; Fernandes et al. 2014). Julliand et al. (2018) reported that horses fed dehydrated alfalfa pellets had higher abundance of amylolytic-utilizing, pectinolytic-utilizing, and lactic acid-utilizing bacteria than those fed sunflower meal pellets.

Hansen et al. (2015) explored the microbial stability, diversity and functionality of equines fed hay (low-nutrient diet) or a mixture of hay and whole oats (high-nutrient diet) as experimental diets and the effects of these diets on their caecal microbial flora. The two diets significantly affected caecal fermentation parameters and bacterial microflora. Low-nutrient diets were more conducive to the diversity and stability of caecal microbiota, which is related to the stabilizing effect of microbial diversity. By contrast, however, high-nutrient diets altered the caecal microbiota composition. In conclusion, hay types influence the structure of microbial communities, fermentation parameters and bacterial abundance.

5.3 | Weaning Method

Weaning causes sudden changes in the diet and patterns, and whether the gut microbiota has a prominent role in regulating physiological responses in equines during weaning stress remains uncertain. Mach et al. (2017) assessed the effects of gradual or abrupt weaning methods on the gut microbiota composition in six age groups (30 days before birth; 0, 3, 5, 7 and 30 days). The results revealed that Prevotella, Paraprevotella and Ruminococcus were more abundant in the abrupt group than in the gradual group. The study revealed that irrespective of the method used, weaning affects the gut microbiota composition of foals. By contrast, Lindenberg et al. (2019) observed that weaning had no effect on the foal microbial composition. They found that the gene expression of pro- and anti-inflammatory cytokines in the blood exhibited no significant change before and after weaning, and the gut microbiota reached a relatively stable level within 50 days of postpartum. The discrepancy in results may be observed because foals are usually weaned at 4-6 months of age, but the transition from milk to solids is gradual, as most foals start consuming solids before weaning.

Diet and nutritional management are crucial factors that influence the gut microbiome, especially in equines. Different dietary components and forage types can significantly alter the structure and function of the gut microbiota, which in turn affects animal health and behaviour (Table 4). Weaning methods may also exert an effect on the gut microbiota of foals. However, this effect may vary according to individual differences. Therefore, careful consideration and management of the diet and nutritional intake of animals is required to maintain their health.

6 | Effects of Disease on the Gut Microbiome

6.1 | Colitis

Equines are typical monogastric herbivores and primarily use crude fibre in their guts for substrate fermentation. Disturbances in the gut microbiota composition or structure cause many diseases in horses, most notably colitis in foals. When the faecal microbiota of healthy horses were compared with those having undifferentiated colitis, *Firmicutes* (68%), *Bacteroidetes* (14%) and *Proteobacteria* (10%) were found to predominate in healthy horses. *Bacteroidetes* (40%), *Firmicutes* (30%) and *Proteobacteria* (18%) were the most abundant in colitis horses (Costa et al. 2012). Garber et al. (2020) also revealed that *Bacteroidetes* was the most abundant in the intestinal tract of colitis horses, and

Fusobacteria was also more abundant in colitis horses than in healthy horses. Alterations in the dominant gut bacterial species are associated with colitis; however, mechanisms linking the gut microbial diversity to colitis need to be elucidated (Costa et al. 2012; Desrochers et al. 2005; Uzal and Diab 2015). The aforementioned studies have shown that colitis is not caused by a single pathogen but is rather a gut-associated disease and is caused by major disturbances in the gut microbiota. In colitis, the number of beneficial bacteria decreases, whereas that of harmful bacteria increases, and this imbalance leads to intestinal inflammation and diarrhoea.

6.2 | Laminitis

Many factors could cause clinical laminitis in horses (Moreau et al. 2014; Milinovich et al. 2010), such as excessive starch intake, excessive fructan intake associated with overgrazing lush pastures and glucocorticoid use (Johnson et al. 2002). Colic can also result in an episode of laminitis. Steelman et al. (2012) performed pyrosequencing of genes of bacteria isolated from faecal samples to characterize the hindgut bacterial community of healthy horses and horses with chronic laminitis. They found that the bacterial community was dominated by Firmicutes (69.21% healthy horses, 56.72% laminitis horses) and Verrucomicrobia (18.13% healthy horses, 27.63% laminitis horses). More operational taxonomic units (OTUs) were observed in the laminitis horse group than in the healthy horse group, and differences were observed in the abundance of Clostridium spp. The most abundant genera in the laminitis horse group were Streptococcus, Clostridium and Treponema; the core flora of the hindgut was Streptococcus. These findings act as a basis for future research on the occurrence and development of chronic laminitis.

6.3 | Parasites and Anthelmintics

Grazing horses may be infected by parasite colonies, mainly nematodes (Ogbourne 1976; Bucknell et al. 1995; Corning 2009; Kuzmina et al. 2016). Parasites present in the gut alter the activity and composition of the gut microbiota (Midha et al. 2017; Peachey et al. 2017). In an experimental pasture trial conducted using 20 parasite-free female Welsh ponies (10 parasite-susceptible and 10 parasite-resistant), the ponies displayed different microbial compositions under parasite-free conditions, and parasitesusceptible ponies exhibited a reduction in the abundance of bacteria such as Ruminococcus, Clostridium XIVa and members of the Lachnospira family (Clark et al. 2018). This may be due to the disruption of mucosal homeostasis on Day 92 of grazing. According to this hypothesis, an increase in pathogenic organisms such as Pseudomonas and Campylobacter, as well as several predicted changes in immune pathways, including pathogen infection and activation of lipid metabolism and signal transduction, are crucial for regulating the immune system and maintaining energy stability. Intestinal parasites can easily cause malnutrition, weight loss, developmental disorders, and so forth, thereby inducing physical damage to the intestine and causing alterations in the gastrointestinal microbial flora. The main factors that cause parasitic infections also need to be explored further.

TABLE 4 | Effects of nutritional and management on the gut microbiota of equines.

	Fibre and starch		Hay types		Weaning method	
Impact	High-fibre diet	High-starch diet	Concentrated diet	Grass diet	Abrupt weaning	Gradual weaning
Key microbial changes	Increased Bacteroidetes, cellulose/xylan degraders	Increased Lactobacillus, Streptococcus	Higher Bacteroidetes in colonic samples	Higher Fibrobacteres and Lactobacil- laceae	Increased abundance of Prevotella, ParaPrevotella, Ruminococcus	Lower levels of Prevotella and Ruminococcus
Microbiome changes	Supports beneficial gut bacteria, improved stability	Increased lactic acid and VFAs production, lower pH in hindgut	Lower fibre-degrading bacteria	Higher diversity in gut microbiota	Reduced microbial stability	Gradual microbiota stabilization
Behavioral/ Gastrointestinal effects	Reduced lactobacilli, better gastrointestinal stability	Potential acidosis, disturbed gut flora and behaviour changes	Altered microbial composition	Reduced acidification, better fibre degradation	Higher pro- inflammatory cytokines	Lower cytokine responses

Abbreviation: VFAs, volatile fatty acids.

In horses, deworming treatment has been suggested as a possible contributor to colic (Hillyer et al. 2002), possibly because this treatment induces an alteration in the gastrointestinal microbiota. However, this conclusion needs to be considered more cautiously, as no relevant study has clearly confirmed that deworming treatment directly causes colic. In some studies, the relative abundance of Bacteroides in the gut reduced in the anthelmintics fenbendazole-treated horses (Sirois 2013). Walshe et al. (2019) demonstrated that the relative abundance of Bacteroides in the gut decreased, irrespective of any age of the horse and the class of therapeutic drugs used (fenbendazole or moxifloxacin). Similar reports have found that the abundance of Bacteroidetes in the equine gut was related to nematode infection, whereas that of Firmicutes was the highest after treatment with fenbendazole for 5 days and ivermectin for 1 day, followed by that of Bacteroidetes (the number of Bacteroidetes displayed a downward trend) (Garber et al. 2020; Walshe et al. 2019). Antihelmintics directly affected the bacterial population, and parasites may change mucin production, thus possibly altering bacterial nutrient milieu in the gut (Fricke et al. 2015). Although the effects of deworming drugs on gut microbiology have been reported, whether deworming directly triggers abdominal pain or changes in the microbiota remains poorly documented. Kunz et al. (2019) demonstrated that moxictin and praziquantel decreased microbial diversity, whereas β -diversity remained unchanged. Although they found significant differences in the abundances of 21 taxonomic groups, they did not believe that deworming treatment majorly affected the gastrointestinal microbiota. In a previous study (Daniels et al. 2017), although bacterial diversity was not significantly altered by moxidectin, 13 different OTUs differed between the experimental and control groups. Although anthelmintics drugs have a limited effect on the gut microflora, they can reduce microbial diversity and abundance. In addition, the duration of drug use is an influencing factor, and thus, medication time should be reasonably controlled. Thus, although deworming treatments may alter the gut microbiota composition to some extent, further studies are warranted to

verify whether this results in abdominal pain or significant gastrointestinal reactions.

6.4 | Obesity and Metabolic Syndrome

Obesity in horses has become increasingly common because of modern management practices and the availability of highenergy feeds. Obese horses exhibit a more diverse intestinal microbial composition and higher microbial abundance than healthy horses (Biddle et al. 2018; Morrison et al. 2018). However, Morrison et al. (2018) reported that the number of Bacteroidetes and Actinomyces increased in the obesity group, whereas Biddle et al. (2018) reported a decrease in these taxa. These differences may be observed due to a more heterogeneous population and a diverse diet in the latter study (Biddle et al. 2018). On the other hand, Morrison et al. (2018) designed the experiment more tightly. Biddle et al. (Zhu et al. 2021) indicated that specific bacterial species may act as markers of obesity and thinness in horses, particularly bacterial groups positively correlated with the blood analysis results and obesity, including Butyrivibrio, Prevotella, Blautia spp., Erysipelotrichaceae and Lachnospiraceae. After the horses lost weight, the relative abundances of Firmicutes and Tenericutes in the gut decreased significantly, whereas that of Bacteroidetes did not change significantly. Although this study did not control diet, revealing metabolic differences between the gut communities of obese and lean horses and designing targeted microbial intervention strategies are essential (Morrison et al. 2018). Thus, microbiota may play a crucial role in the physiology of weight regulation (Garber et al. 2020). Faecal transplantation into the gut is recommended to further study the gut microbial composition and determine if similar effects exist.

In addition, Elzinga et al. (2016) revealed differences in the overall microflora composition between equine metabolic syndrome (EMS) horses and healthy horses. According to the results, the abundances of *Clostridium* cluster XI, *Verrucobacterium*

TABLE 5 | Effects of disease on the intestinal flora of equines.

Disease type	Microbiome changes	Impact	
Colitis	Increase in <i>Bacteroidetes</i> and <i>Proteobacteria</i> , decrease in <i>Firmicutes</i>	intestinal inflammation and diarrhoea	
Laminitis	Streptococcus, Clostridium and Treponema	Laminitis development	
Parasites and anthelmintics	Altered activity and composition of gut microbiota	Malnutrition, weight loss, developmental disorders	
Obesity and metabolic syndrome	More diverse and abundant gut microbiota	Weight regulation and metabolic differences	

and *Lactobacillaceae* bacteria increased, whereas those of *Lachnospiraceae*, *Flavobacteriaceae* and *Rhodospirillaceae* decreased. Another study also identified the faecal microbiota composition of EMS horses and healthy controls. EMS horses exhibited a decreased microbial diversity, but whether lean and obese horses with the same diet will have similar differences remains unclear (Garber et al. 2020).

Overall, diseases such as colitis and laminitis as well as conditions like parasitic infections and obesity significantly alter the gut microbiome of horses (Table 5). These changes can lead to a range of health problems, highlighting the importance of maintaining a balanced gut microbiome for equine health. Further research is warranted to fully understand the complex interactions among gut health, disease and microbiome balance.

7 | Conclusion

The animal gastrointestinal tract comprises a large and diverse microbial community. This community not only affects nutrient digestion and absorption but also participates in regulating many physiological functions of the host, including disease occurrence. The symbiosis between the host and microbes can promote animal health, thereby benefiting and improving animal productivity. In the case of horses, attention must be paid to the external environment, and their rational management must be strengthened in combination with the characteristics of this species, such as providing a reasonable diet, managing weaning method and time, preventing parasitic infections and using deworming drugs rationally. Moreover, a large research gap exists in the deep signal transduction mechanism, and whether the dominant factor affecting the microbial community structure is the diet or host genotype remains uncertain. Therefore, several in-depth studies on intestinal microbes must be conducted, as they would offer crucial theoretical guidance for improving the gastrointestinal microecological environment of horses and donkeys and preventing and controlling disease occurrence.

Author Contributions

Wei Zhang and Rong Guo wrote the manuscript, Ablat Sulayman and Yujiang Sun provided guidance, Shuqin Liu reviewed and edited it. All authors have read and agreed to the published version of the manuscript.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

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Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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References

Ang, L., G. Vinderola, A. Endo, et al. 2022. "Gut Microbiome Characteristics in Feral and Domesticated Horses From Different Geographic Locations." *Communications Biology* 5, no. 1: 172.

Bäckhed, F., J. Roswall, Y. Peng, et al. 2015. "Dynamics and Stabilization of the Human Gut Microbiome During the First Year of Life." *Cell Host & Microbe* 17, no. 5: 690–703.

Bergman, E. 1990. "Energy Contributions of Volatile Fatty Acids From the Gastrointestinal Tract in Various Species." *Physiological Reviews* 70, no. 2: 567–590.

Biddle, A. S., J. F. Tomb, and Z. Fan. 2018. "Microbiome and Blood Analyte Differences Point to Community and Metabolic Signatures in Lean and Obese Horses." *Frontiers in Veterinary Science* 5: 225.

Broom, D. M. 2006. "Behaviour and Welfare in Relation to Pathology." *Applied Animal Behaviour Science* 97, no. 1: 73–83.

Bucknell, D., R. Gasser, and I. Beveridge. 1995. "The Prevalence and Epidemiology of Gastrointestinal Parasites of Horses in Victoria, Australia." *International Journal for Parasitology* 25, no. 6: 711–724.

Chaucheyras-Durand, F., A. Sacy, K. Karges, and E. Apper. 2022. "Gastro-Intestinal Microbiota in Equines and Its Role in Health and Disease: The Black Box Opens." *Microorganisms* 10, no. 12: 2517.

Clark, A., G. Sallé, V. Ballan, et al. 2018. "Strongyle Infection and Gut Microbiota: Profiling of Resistant and Susceptible Horses Over a Grazing Season." *Frontiers in Physiology* 9: 272.

Corning, S. 2009. "Equine Cyathostomins: A Review of Biology, Clinical Significance and Therapy." *Parasites Vectors* 2, no. 2: 1–6.

- Costa, M. C., G. Silva, R. V. Ramos, et al. 2015. "Characterization and Comparison of the Bacterial Microbiota in Different Gastrointestinal Tract Compartments in Horses." *Veterinary Journal* 205, no. 1: 74–80.
- Costa, M. C., L. G. Arroyo, E. Allen-Vercoe, et al. 2012. "Comparison of the Fecal Microbiota of Healthy Horses and Horses With Colitis by High Throughput Sequencing of the V3–V5 Region of the 16S rRNA Gene." *PLoS ONE* 7, no. 7: e41484.
- Costa, M., H. Stämpfli, E. Allen-Vercoe, and J. S. Weese. 2016. "Development of the Faecal Microbiota in Foals." *Equine Veterinary Journal* 48, no. 6: 681–688.
- Daly, K., C. J. Proudman, S. H. Duncan, H. J. Flint, J. Dyer, and S. P. Shirazi-Beechey. 2012. "Alterations in Microbiota and Fermentation Products in Equine Large Intestine in Response to Dietary Variation and Intestinal Disease." *British Journal of Nutrition* 107, no. 7: 989–995
- Daniels, S., J. Leng, R. Ellis, J. Swann, M. Moore-Colyer, and C. Proudman. 2017. "The Effect of Moxidectin Treatment on the Equine Hind Gut Microbiome, Metabonome and Feed Fermentation Kinetics in Horses With Very Low Parasite Burdens." *Equine Veterinary Education* 29, no. 58: 6–6.
- De Filippo, C., D. Cavalieri, M. Di Paola, et al. 2010. "Impact of Diet in Shaping Gut Microbiota Revealed by a Comparative Study in Children From Europe and Rural Africa." *Proceedings of the National Academy of Sciences* 107, no. 33: 14691–14696.
- De Fombelle, A., V. Julliand, C. Drogoul, and E. Jacotot. 2001. "Feeding and Microbial Disorders in Horses: 1-Effects of an Abrupt Incorporation of Two Levels of Barley in a Hay Diet on Microbial Profile and Activities." *Journal of Equine Veterinary Science* 21, no. 9: 439–445.
- De La, T. U., J. D. Henderson, K. L. Furtado, et al. 2019. "Utilizing the Fecal Microbiota to Understand Foal Gut Transitions From Birth to Weaning." *PLoS ONE* 14, no. 4: e0216211.
- Desrochers, A. M., B. A. Dolente, M.-F. Roy, R. Boston, and S. Carlisle. 2005. "Efficacy of *Saccharomyces boulardii* for Treatment of Horses With Acute Enterocolitis." *Journal of the American Veterinary Medical Association* 227, no. 6: 954–959.
- Destrez, A., P. Grimm, F. Cézilly, and V. Julliand. 2015. "Changes of the Hindgut Microbiota due to High-Starch Diet Can be Associated With Behavioral Stress Response in Horses." *Physiology Behavior* 149: 159–164.
- Dicks, L., M. Botha, E. Dicks, and M. Botes. 2014. "The Equine Gastro-Intestinal Tract: An Overview of the Microbiota, Disease and Treatment." *Livestock Science* 160: 69–81.
- Dougal, K., G. de la Fuente, P. A. Harris, et al. 2014. "Characterisation of the Faecal Bacterial Community in Adult and Elderly Horses Fed a High Fibre, High Oil or High Starch Diet Using 454 Pyrosequencing." *PLoS ONE* 9, no. 2: e87424.
- Elia, J. B., H. N. Erb, and K. A. Houpt. 2010. "Motivation for Hay: Effects of a Pelleted Diet on Behavior and Physiology of Horses." *Physiology Behavior* 101, no. 5: 623–627.
- Elzinga, S. E., J. S. Weese, and A. A. Adams. 2016. "Comparison of the Fecal Microbiota in Horses With Equine Metabolic Syndrome and Metabolically Normal Controls Fed a Similar All-Forage Diet." *Journal of Equine Veterinary Science* 44: 9–16.
- Ericsson, A. C., P. J. Johnson, M. A. Lopes, S. C. Perry, and H. R. Lanter. 2016. "A Microbiological Map of the Healthy Equine Gastrointestinal Tract." *PLoS ONE* 11, no. 11: e0166523.
- Faubladier, C., S. Sadet-Bourgeteau, C. Philippeau, E. Jacotot, and V. Julliand. 2014. "Molecular Monitoring of the Bacterial Community Structure in Foal Feces Pre-and Post-Weaning." *Anaerobe* 25: 61–66.
- Fernandes, K. A., S. Kittelmann, C. W. Rogers, et al. 2014. "Faecal Microbiota of Forage-Fed Horses in New Zealand and the Population Dynamics of Microbial Communities Following Dietary Change." *PLoS ONE* 9, no. 11: e112846.

- Fricke, W. F., Y. Song, A.-J. Wang, et al. 2015. "Type 2 Immunity-Dependent Reduction of Segmented Filamentous Bacteria in Mice Infected With the Helminthic Parasite *Nippostrongylus brasiliensis*." *Microbiome* 3, no. 1: 1–12.
- Garber, A., P. Hastie, and J.-A. Murray. 2020. "Factors Influencing Equine Gut Microbiota: Current Knowledge." *Journal of Equine Veterinary Science* 88: 102943.
- Garner, H., D. Hutcheson, J. Coffman, A. Hahn, and C. Salem. 1977. "Lactic Acidosis: A Factor Associated With Equine Laminitis." *Journal of Animal Science* 45, no. 5: 1037–1041.
- Goodson, J., W. Tyznik, J. Cline, and B. Dehority. 1988. "Effects of an Abrupt Diet Change From Hay to Concentrate on Microbial Numbers and Physical Environment in the Cecum of the Pony." *Applied Environmental Microbiology* 54, no. 8: 1946–1950.
- Hansen, N. C., E. Avershina, L. T. Mydland, et al. 2015. "High Nutrient Availability Reduces the Diversity and Stability of the Equine Caecal Microbiota." *Microbial Ecology in Health Disease* 26, no. 1: 27216.
- Harris, P. A., A. D. Ellis, M. J. Fradinho, et al. 2017. "Review: Feeding Conserved Forage to Horses: Recent Advances and Recommendations." *Animal* 11, no. 6: 958–967.
- Harris, P., and C. Dunnett. 2018. "Nutritional Tips for Veterinarians." *Equine Veterinary Education* 30, no. 9: 486–496.
- Hillyer, M., F. Taylor, C. Proudman, G. Edwards, J. Smith, and N. French. 2002. "Case Control Study to Identify Risk Factors for Simple Colonic Obstruction and Distension Colic in Horses." *Equine Veterinary Journal* 34, no. 5: 455–463.
- Johnson, P. J., S. H. Slight, V. K. Ganjam, and J. M. Kreeger. 2002. "Glucocorticoids and Laminitis in the Horse." *Veterinary Clinics: Equine Practice* 18, no. 2: 219–236.
- Juśkiewicz, J., B. Fotschki, J. Jaworska, and M. Siemieniuch. 2021. "Investigations of the Maintenance System of the Konik Polski Horse and Its Effects on Fecal Microbiota Activity During the Winter and Summer Seasons." *Animal Science Journal = Nihon Chikusan Gakkaiho* 92, no. 1: e13603.
- Julliand, S., A. Martin, and V. Julliand. 2018. "Effect of Dehydrated Alfalfa on Equine Gastric and Faecal Microbial Ecosystems." *Livestock Science* 215: 16–20.
- Kauter, A., L. Epping, T. Semmler, et al. 2019. "The Gut Microbiome of Horses: Current Research on Equine Enteral Microbiota and Future Perspectives." *Animal Microbiome* 1, no. 1. https://doi.org/10.1186/s42523-019-0013-3
- Kunz, I. G., K. J. Reed, J. L. Metcalf, et al. 2019. "Equine Fecal Microbiota Changes Associated With Anthelmintic Administration." *Journal of Equine Veterinary Science* 77: 98–106.
- Kuzmina, T. A., I. Dzeverin, and V. A. Kharchenko. 2016. "Strongylids in Domestic Horses: Influence of Horse Age, Breed and Deworming Programs on the Strongyle Parasite Community." *Veterinary Parasitology* 227: 56–63.
- Langner, K., and I. Vervuert. 2019. "Impact of Nutrition and Probiotics on the Equine Microbiota: Current Scientific Knowledge and Legal Regulations." *Tierarztliche Praxis. Ausgabe G, Grosstiere Nutztiere* 47, no. 1: 35–48.
- Lara, F., R. Castro, and P. Thomson. 2022. "Changes in the Gut Microbiome and Colic in Horses: Are They Causes or Consequences?" *Open Veterinary Journal* 12, no. 2: 242–249.
- Li, Y., Q. Ma, X. Shi, G. Liu, and C. Wang. 2022. "Integrated Multi-Omics Reveals Novel Microbe-Host Lipid Metabolism and Immune Interactions in the Donkey Hindgut." *Frontiers in Immunology* 13: 1003247.
- Lindenberg, F., L. Krych, W. Kot, et al. 2019. "Development of the Equine Gut Microbiota." *Scientific Reports* 9, no. 1: 1–9.
- Liu, G., G. Bou, S. Su, et al. 2019. "Microbial Diversity Within the Digestive Tract Contents of Dezhou Donkeys." *PLoS ONE* 14, no. 12: e0226186.

- Liu, H., X. Zhao, X. Han, et al. 2020. "Comparative Study of Gut Microbiota in Tibetan Wild Asses (Equus kiang) and Domestic Donkeys (Equus asinus) on the Qinghai-Tibet Plateau." *PeerJ* 8: e9032.
- Lowden, S., and T. Heath. 1995. "Segmented Filamentous Bacteria Associated With Lymphoid Tissues in the Ileum of Horses." *Research in Veterinary Science* 59, no. 3: 272–274.
- Mach, N., A. Foury, S. Kittelmann, et al. 2017. "The Effects of Weaning Methods on Gut Microbiota Composition and Horse Physiology." *Frontiers in Physiology* 8: 535.
- Medina, B., I. Girard, E. Jacotot, and V. Julliand. 2002. "Effect of a Preparation of Saccharomyces Cerevisiae on Microbial Profiles and Fermentation Patterns in the Large Intestine of Horses Fed a High Fiber or a High Starch Diet." *Journal of Animal Science* 80, no. 10: 2600–2609.
- Midha, A., J. Schlosser, and S. Hartmann. 2017. "Reciprocal Interactions Between Nematodes and Their Microbial Environments." *Frontiers in Cellular Infection Microbiology* 7: 144.
- Milinovich, G. J., A. V. Klieve, C. C. Pollitt, and D. J. Trott. 2010. "Microbial Events in the Hindgut During Carbohydrate-Induced Equine Laminitis." *Veterinary Clinics: Equine Practice* 26, no. 1: 79–94.
- Moreau, M. M., S. C. Eades, C. R. Reinemeyer, M. N. Fugaro, and J. C. Onishi. 2014. "Illumina Sequencing of the V4 Hypervariable Region 16S rRNA Gene Reveals Extensive Changes in Bacterial Communities in the Cecum Following Carbohydrate Oral Infusion and Development of Early-Stage Acute Laminitis in the Horse." *Journal of Equine Veterinary Science* 168, no. 2–4: 436–441.
- Morrison, P. K., C. J. Newbold, E. Jones, et al. 2018. "The Equine Gastrointestinal Microbiome: Impacts of Age and Obesity." *Frontiers in Microbiology* 9: 3017.
- Ogbourne, C. P. 1976. "The Prevalence, Relative Abundance and Site Distribution of Nematodes of the Subfamily Cyathostominae in Horses Killed in Britain." *Journal of Helminthology* 50, no. 3: 203–214.
- Peachey, L. E., T. P. Jenkins, and C. Cantacessi. 2017. "This Gut Ain't Big Enough for Both of Us. Or Is It? Helminth–Microbiota Interactions in Veterinary Species." *Trends in Parasitology* 33, no. 8: 619–632.
- Quercia, S., F. Freccero, C. Castagnetti, et al. 2019. "Early Colonisation and Temporal Dynamics of the Gut Microbial Ecosystem in Standardbred Foals." *Equine Veterinary Journal* 51, no. 2: 231–237.
- Reed, K. J., I. G. Z. Kunz, J. A. Scare, et al. 2021. "The Pelvic Flexure Separates Distinct Microbial Communities in the Equine Hindgut." *Scientific Reports* 11, no. 1: 4332.
- Reed, K., I. Kunz, J. Scare, et al. 2017. "Can Fecal Samples be Used to Inform About Microbial Communities of the Equine Hindgut?" *Journal of Equine Veterinary Science* 100, no. 52: 52–53.
- Richards, N., G. Hinch, and J. B. Rowe. 2006. "The Effect of Current Grain Feeding Practices on Hindgut Starch Fermentation and Acidosis in the Australian Racing Thoroughbred." *Australian Veterinary Journal* 84, no. 11: 402–407.
- Santos, A. S., M. A. Rodrigues, R. J. Bessa, L. M. Ferreira, and W. Martin-Rosset. 2011. "Understanding the Equine Cecum-Colon Ecosystem: Current Knowledge and Future Perspectives." *Animal* 5, no. 1: 48–56.
- Sirois, R. J. 2013. "Comparison of the Fecal Microbiota of Horses Before and After Treatment for Parasitic Helminths: Massively Parallel Sequencing of the v4 Region of the 16S Ribosomal RNA Gene." Masters Thesis, Smith College.
- Sorensen, R. J., J. S. Drouillard, T. L. Douthit, et al. 2021. "Effect of Hay Type on Cecal and Fecal Microbiome and Fermentation Parameters in Horses." *Journal of Animal Science* 99, no. 1: skaa407.
- Steelman, S. M., B. P. Chowdhary, S. Dowd, J. Suchodolski, and J. E. Janecka. 2012. "Pyrosequencing of 16S rRNA Genes in Fecal Samples Reveals High Diversity of Hindgut Microflora in Horses and Potential Links to Chronic Laminitis." *BMC Veterinary Researc* 8: 231.

- Su, S., Y. Zhao, Z. Liu, et al. 2020. "Characterization and Comparison of the Bacterial Microbiota in Different Gastrointestinal Tract Compartments of Mongolian Horses." *Microbiologyopen* 9, no. 6: 1085–1101.
- Uzal, F. A., and S. S. Diab. 2015. "Gastritis, Enteritis, and Colitis in Horses." *Veterinary Clinics: Equine Practice* 31, no. 2: 337–358.
- Walshe, N., R. Cabrera-Rubio, R. Collins, et al. 2021. "A Multiomic Approach to Investigate the Effects of a Weight Loss Program on the Intestinal Health of Overweight Horses." *Frontiers in Veterinary Science* 8: 668120.
- Walshe, N., V. Duggan, R. Cabrera-Rubio, et al. 2019. "Removal of Adult Cyathostomins Alters Faecal Microbiota and Promotes an Inflammatory Phenotype in Horses." *International Journal for Parasitology* 49, no. 6: 489–500.
- Warzecha, C., J. Coverdale, J. Janecka, et al. 2017. "Influence of Short-Term Dietary Starch Inclusion on the Equine Cecal Microbiome." *Journal of Animal Science* 95, no. 11: 5077–5090.
- Willing, B., A. Vörös, S. Roos, C. Jones, A. Jansson, and J. Lindberg. 2009. "Changes in Faecal Bacteria Associated With Concentrate and Forage-Only Diets Fed to Horses in Training." *Equine Veterinary Journal* 41, no. 9: 908–914.
- Wu, G. D., J. Chen, C. Hoffmann, et al. 2011. "Linking Long-Term Dietary Patterns With Gut Microbial Enterotypes." *Science* 334, no. 6052: 105–108.
- Wunderlich, G., M. Bull, T. Ross, M. Rose, and B. Chapman. 2023. "Understanding the Microbial Fibre Degrading Communities & Processes in the Equine Gut." *Animal Microbiome* 5, no. 1. https://doi.org/10.1186/s42523-022-00224-6
- Xing, J., G. Liu, X. Zhang, et al. 2020. "The Composition and Predictive Function of the Fecal Microbiota Differ Between Young and Adult Donkeys." *Frontiers in Microbiology* 11: 596394.
- Zaitseva, S., O. Dagurova, A. Radnagurueva, et al. 2023. "Fecal Microbiota and Diet Composition of Buryatian Horses Grazing Warm- and Cold-Season Grass Pastures." *Microorganisms* 11, no. 8: 1947.
- Żak-Bochenek, A., P. Żebrowska-Różańska, J. Bajzert, et al. 2024. "Comparison and Characterization of the Bacterial Microbiota and SIgA Production in Different Gastrointestinal Segments in Horses." *Veterinary Research Communications* 48, no. 6: 3605–3620.
- Zhao, Y., B. Li, D. Bai, et al. 2016. "Comparison of Fecal Microbiota of Mongolian and Thoroughbred Horses by High-Throughput Sequencing of the V4 Region of the 16S rRNA Gene." *Asian-Australasian Journal of Animal Sciences* 29, no. 9: 1345–1352.
- Zhu, Y., X. Wang, B. Liu, et al. 2021. "The Effect of Ryegrass Silage Feeding on Equine Fecal Microbiota and Blood Metabolite Profile." *Frontiers in Microbiology* 12: 715709.