

The Microbiota of the Equine Gut and what it can tell us about feeding horses

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Excerpted from the upcoming book, *Through the Looking-Glass: a bug's-eye view of the equine gut, and what it can tell us about feeding horses.*

Key points

1. Bacteria predominate, but the equine gut microbiota also includes archaea, protozoa, fungi, and viruses.
2. There are an estimated 2–4 quadrillion bacteria in the healthy equine gut; 99% are found in the hindgut (caecum and large colon).
3. There are > 10,000 different bacterial species/strains and > 500 different fungal species *per* horse in the healthy equine hindgut.
4. The gut microbiota is unique to each horse, is not entirely knowable as it is not entirely fixed (static), and is highly conserved even in the face of massive challenge.
5. The gut microbiota is established in the first 1–2 months of life (when coprophagy peaks), being most similar to the foal's dam than to any other horse; thereafter, it resists much change.
6. Microbial diversity is an important feature of the healthy equine gut; healthy and well-performing horses generally have greater diversity than their ill or poor-performing counterparts.
7. Microbial diversity is facilitated by a natural diet, which for horses is high in fibre, low in starch, and comprises a diversity of plant types and parts that change with the season.
8. When natural grazing is unavailable or inadvisable, feeding a variety of hays and herbs can add diversity to a compositionally limited diet.

Definitions

Microbiota — the microbial community, comprising bacteria, archaea, protozoa, fungi, and viruses

Microbiome — the entire ecosystem in which the microbiota exists

CFU — colony-forming unit; equivalent to a single live microbe

OTU — operational taxonomic unit; equivalent to a single microbial species

ASV — amplicon (gene) sequence variant; equivalent to a single species or strain (variant, subspecies)

Richness — number of different species in a community

Evenness — relative abundance of the different species in a community

Diversity — an expression of the richness and evenness of a community

1. Bacteria predominate

The microbiota of the healthy equine gut is dominated by bacteria. While fewer in number and more obscure in function, the microbiota also comprises archaea, eukaryotes (protozoa, fungi), and viruses (Fig. 1).¹

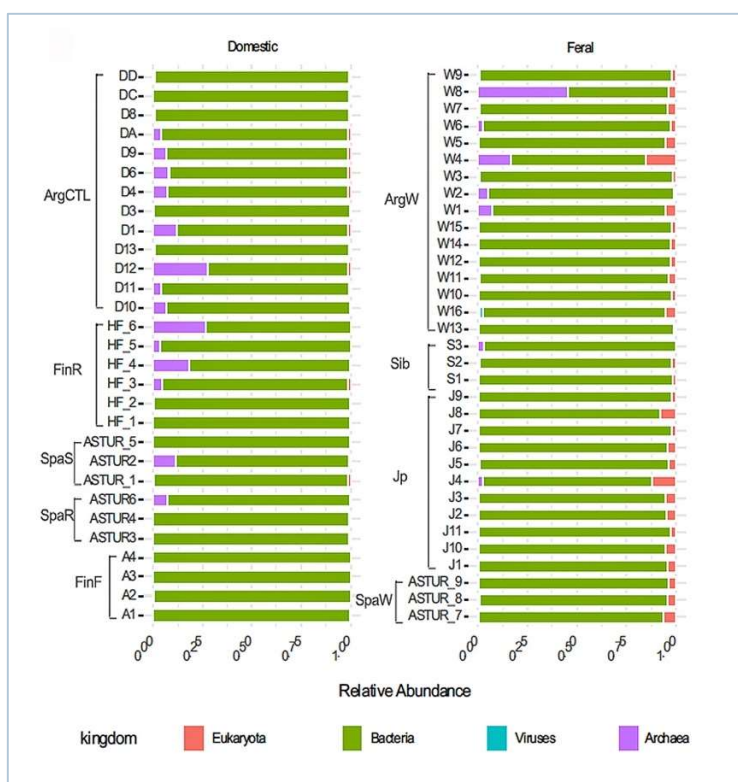


Figure 1. Faecal microbiota of 57 horses (domestic and feral) in 5 countries (Argentina, Finland, Spain, Russia, Japan) on 3 continents. At the kingdom level, Bacteria predominate (70–99%).¹

We know precious little about the gut bacteria in horses — we can't even identify which phylum some OTUs (species) belong to, which is a bit like not being able to identify a horse as a vertebrate! We know even less about these other taxa. Almost all of the research on the equine gut microbiota has focused on the bacteria, and on naming them, like pinning dead butterflies to a corkboard and assigning them scientific labels.

Most of what we know about the functioning of the equine gut microbiota is (a) assumed from research in other species, particularly the ruminal microbiota in cattle, and (b) implied from gross measurements of feed digestibility or the concentrations of volatile fatty acids (acetate, propionate, butyrate, *etc.*) and other metabolites in fresh faeces, selected gut compartments, or laboratory simulator devices. Most of the microbes identified using genetic methods can't even be cultured for study. So, for all we do know, the microcosmos of the equine gut remains a veritable 'black box'.

Furthermore, the research to date has primarily focused on two aspects: (1) potential pathogens, and (2) how to exploit or manipulate the horse's gut microbiota to our advantage, such as mitigating the harmful effects of a high-starch diet rather than feeding a more natural diet. Research in other species takes things a step further by looking to the gut microbiota for the next generation of antibiotics and other potential drugs. In fact, animal 'biome journals, conferences, and companies openly use the word 'exploit'.

2. Number and distribution of gut bacteria

Based on concentrations in the different gut compartments, and on the capacity of each segment, there are an estimated 2–4 quadrillion bacteria in the healthy equine gut. Estimates for the small and large intestine combined range from 1.8 to 4.4 quadrillion (Table 1).

Table 1. Estimates of bacterial numbers in the intestine of the average horse.

Compartment	Concentration	Total Number*	Relative Number
Small intestine	400–500 million/ml	26–32 trillion	0.9%
Caecum	5–11 billion/ml	170–375 trillion	8.5%
Large colon	20–50 billion/ml	1.6–4 quadrillion	90.6%
TOTAL	–	1.8–4.4 quadrillion	(100%)

* Based on the capacity of each compartment in the average adult horse. Combined, the small and large intestine represents 90% of the length and 85% of the capacity of the equine GI tract.²

For context, a quadrillion is 1 followed by 15 zeroes. If a single bacterium were represented by 1 second, whereas 1 million seconds = 12 days, 1 quadrillion seconds = 32 million years.

Over 90% of the gut bacteria are found in the large colon. Together with the caecum, the hindgut is home to approximately 99% of the equine gut microbiota. Little wonder that almost all of the research on the equine gut microbiota has focused on the hindgut, most often using faeces as a proxy for the large colon (which is mostly valid).

3. Microbial richness — and the problem(s) with probiotics

On average, there are > 10,000 different bacterial ASVs (species/strains) *per* horse in the faeces of healthy horses,³ and collectively > 35,000 different bacterial OTUs/ASVs *per* study group.⁴ As for the fungal community, there are an average of > 500 different fungal OTUs *per* horse⁵ and > 2,100 different fungal OTUs *per* study group.⁶ (The richness of the archaea, protozoa, and viruses that inhabit the equine gut remains poorly characterised, although some attempts have been made.¹)

Granted, not all of these species/strains will have colonised the gut; some will simply be passing through. And not all gene sequence 'reads' represent live organisms. But that brings us to the issue of probiotics.

Independent research on equine probiotic products is underwhelming.

Is it any wonder? Most equine probiotics contain only a few different bacterial species/strains; some contain only one! And none are important species, nor even important genera, in the equine hindgut. Notably, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* are found in extremely low abundance — and often not at all — in the faeces or hindgut of healthy horses.

Figure 2 depicts the main bacterial genera, by compartment, in 11 adult horses without gastrointestinal disease.⁷ *Lactobacillus* predominates only in the stomach and small intestine; it is negligible in the hindgut. *Bifidobacterium* and *Enterococcus* don't rate a mention in any compartment. Also note that many of the hindgut bacteria are unclassified at the genus level (*i.e.*, we don't even know which genus they belong to)!

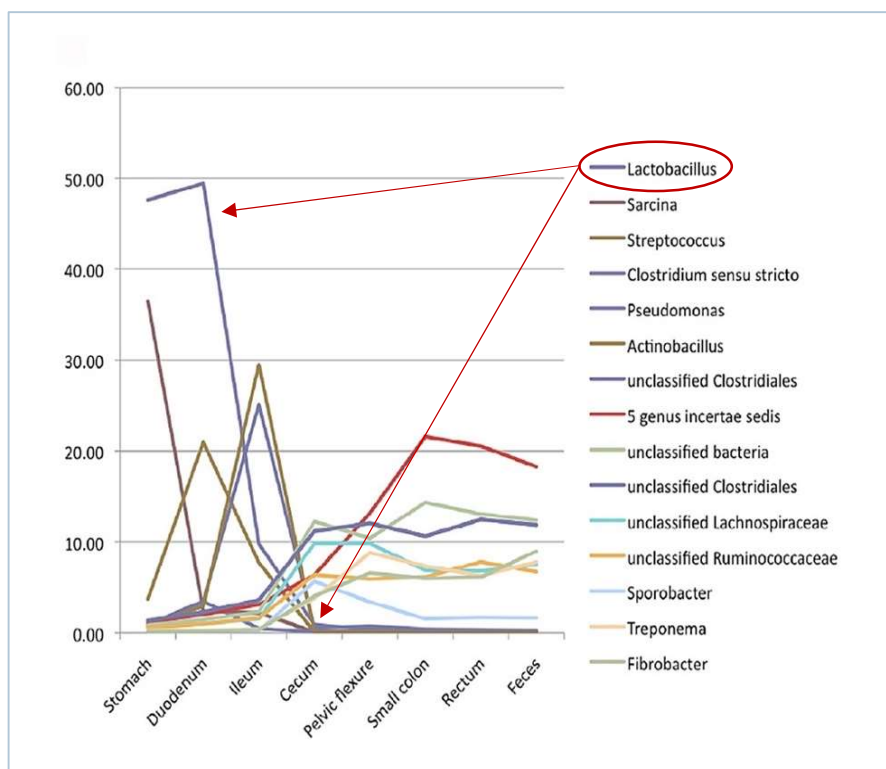


Figure 2. Relative abundance (%) of the main bacterial genera, by compartment, in 11 adult horses.⁷

As to the stomach, the average equine stomach contains anywhere from 80 to > 140 OTUs *per* horse.⁷⁻⁹ And while *Lactobacillus* predominates in many (but not all) studies, the particular species are unfamiliar, such as *L. hayakitensis*, *L. equigenersi*, and 'sister group to *L. jensenii* and *L. fornicalis*'.⁸

To continue, most probiotics contain just a few billion CFUs per dose — and that's simply the label claim; how many are viable and capable of colonisation is another matter. How far can 1 billion, or even 10 billion, CFUs go in a system whose microbiota may number > 4 quadrillion and comprise > 10,000 different species?

As for the rich fungal ecology of the equine gut, the only fungal genus to appear in equine probiotics is *Saccharomyces*, either *S. cerevisiae* or its subspecies *S. boulardii*. Yet this species is not described as part of the equine gut microbiota. For example, in the study comparing the faecal microbiota of domestic and feral horses in 5 countries, *S. pastorianus* was the only representative of this genus present in any meaningful numbers; and its relative abundance in the fungal community ranged from >50% to 0% (Fig. 3, next page).¹

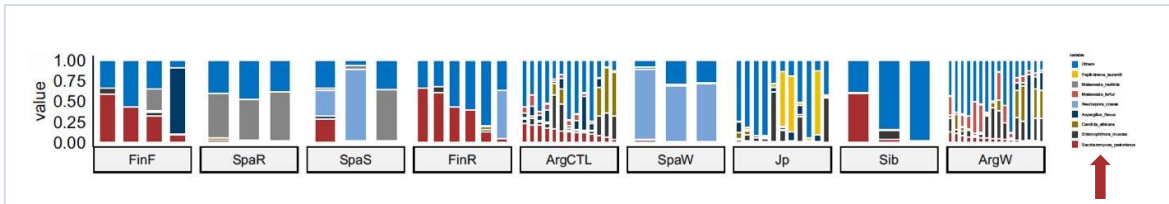


Figure 3. Relative abundance (ratio) of the main fungal genera, by country and setting, in the faeces of 57 horses. *Saccharomyces pastorianus* (dark red) is shown at the bottom of each column, when present.¹

Lastly (for now), commercial probiotic products for horses are adapted from those designed for humans or production animals (ruminants, pigs, poultry). So, it should come as no surprise that equine probiotics have generally been disappointing when studied objectively, showing little or no benefit, and potentially causing harm (e.g., diarrhoea in foals,¹⁰ worsening of clinical signs in horses with gastric ulcers).

4. The microbiota is unique to each horse and is highly conserved

The concept of a 'core' — common, shared, characteristic, 'signature' — gut microbiota is generally accepted and widely applied in microbiota research. It is so pervasive in human and animal studies that it has become a truism in equine gut microbiota research as well.

But is there a 'core' gut microbiota in horses?

In a word, NO, not in any meaningful sense. There are some broad themes at the phylum level, but even at this 'thirty-thousand-foot' perspective, there is a disconcerting inconsistency among studies (Fig. 4).¹¹

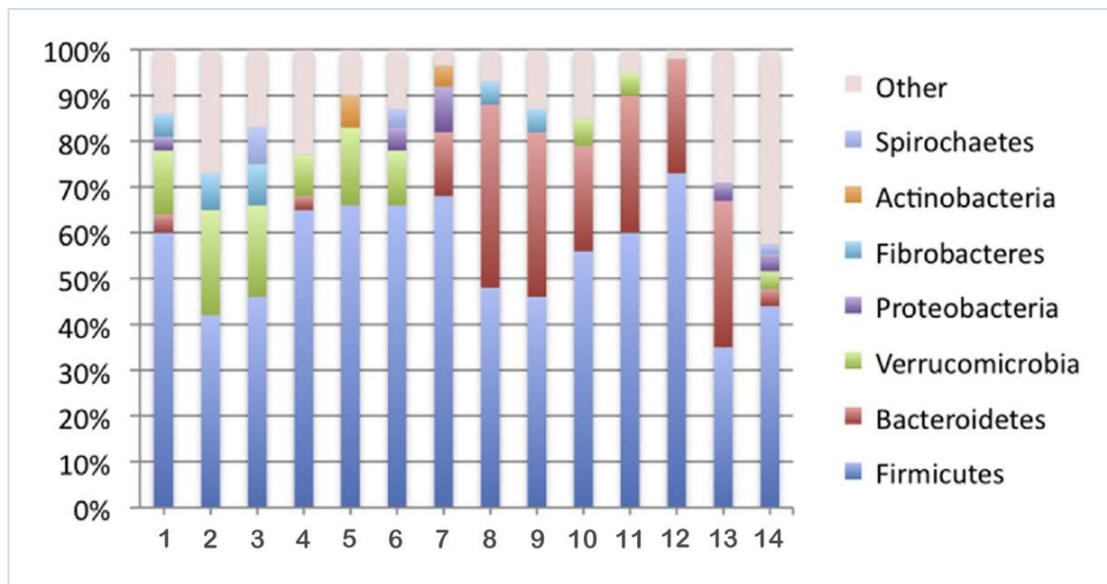


Figure 4. Relative abundance of the main bacterial phyla in the faeces of healthy horses across 14 different studies. *Firmicutes* predominates (>50% abundance) in most, but by no means all, studies. *Bacteroidetes* and *Verrucomicrobia* jostle for second place, and there is no consistency in the Firmicutes:Bacteroidetes (used as a marker of dysbiosis in human and animal studies⁴). The 'Other' category covers a lot of ground, including various low-abundance phyla and the OTUs that cannot be classified even at the phylum level.¹¹

What accounts for this inconsistency? First, there are substantial variations in methodology between the older and newer studies, as advances in this field are rapid and ongoing. But perhaps the bigger 'fly in the ointment' is the little matter of individual variation among horses.

Without meaning to, the research on the gut microbiota in domestic and feral horses, and relatedly the ruminal microbiota in dairy cows, consistently shows these three facts:

1. Each individual has their own unique microbiota that is distinctly different from their herdmates.
2. Each individual's microbiota is not entirely knowable because it is not entirely fixed (static); for example, the faecal microbiota may change by 25% over a single grazing season (Fig. 5).¹²
3. Each individual's microbiota is conserved, even in the face of massive challenge; for example, cows restore their original ruminal microbiota after near-total ruminal exchange (Fig. 6, next page).¹³

These elements are also seen in studies of faecal microbial transfer (FMT) in dogs and cats. For example, a pair of studies on oral FMT in dogs¹⁴ and cats¹⁵ with chronic enteropathy compared the donor and recipient faecal microbiota 2 weeks after completing a course of 50 doses. On average, only 18% of donor ASVs in dogs and 13% in cats “engrafted” in the recipient. Some quotes: “...responses were individual-specific, with host identity accounting for 69% of the variation,”¹⁴ and “FMT recipients are not necessarily gaining taxonomically novel microbes but instead ASVs that are taxonomically similar to those that they start with.”¹⁵

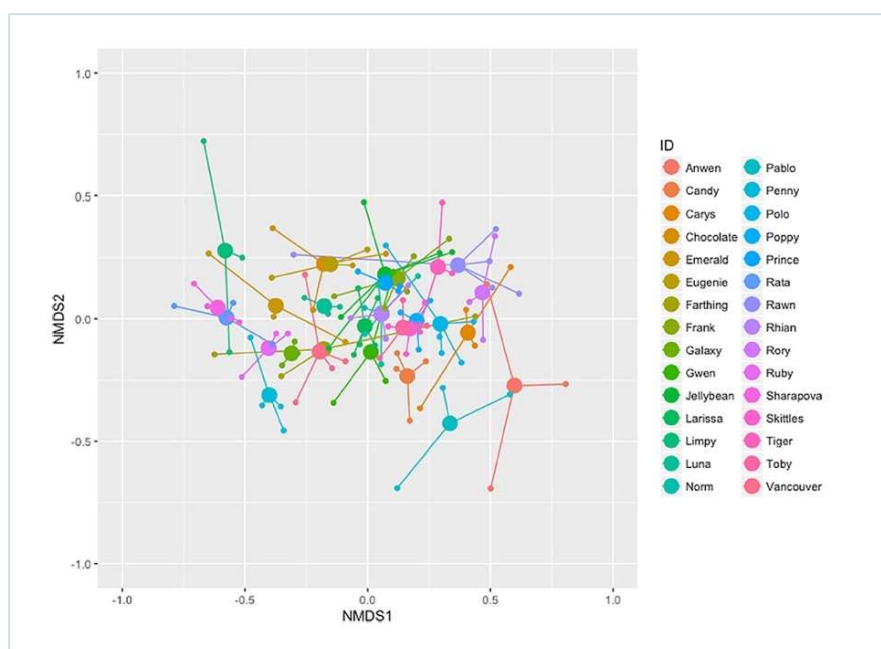


Figure 5. Total faecal microbiota of free-living Welsh Mountain ponies in the Carneddau mountains of Wales over a 3-month period (Aug–Nov). Each colour represents a single pony (n=30). Each small dot represents a separate faecal sample (3–5 per pony). Each large dot represents the calculated average for that pony.¹²

In a study of dairy cows undergoing near-total exchange of their ruminal contents, it took as little as 2 weeks in some, and > 2 months in others, for the cow's original microbiota to be restored.¹³ Regardless of how long it took, and how successful the cow was at fully restoring her ruminal microbiota, all of the cows made concerted efforts to restore their own, original ruminal microbial communities after this massive challenge.

Consider the implications for probiotic use and FMT in horses. How could these interventions change an individual's microbiota long-term when near-total ruminal content exchange could not? Not surprisingly, clinical outcomes for FMT in horses are inconsistent.¹⁶ In fact, in a recent experimental study, an oral carbonate solution was as effective as FMT in restoring the faecal microbiota in horses with oligofructose-induced diarrhoea,¹⁷ which suggests that any effect of FMT is primarily chemical rather than microbiological.

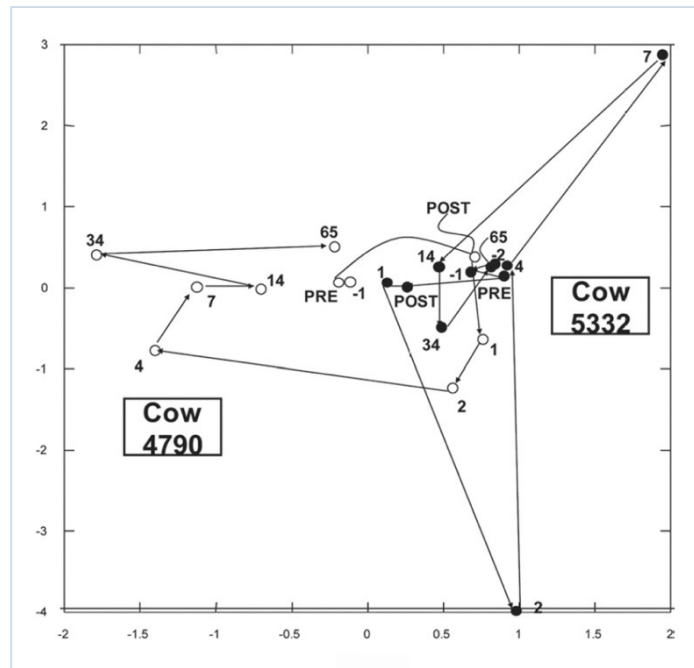


Figure 6. Ruminal bacterial community composition in a pair of lactating dairy cows before and after near-total exchange of their ruminal contents. The small numbers are the sampling times, from the day before (-1), through the day of (PRE and POST), and for up to 65 days after ruminal exchange.¹³

How did those cows manage that trick?

Within each gut compartment, there are two distinct microbial communities:

- luminal — free-floating within the liquid portion of the ingesta/digesta or attached to rafts of solid food material and cell debris
- mucosal — embedded within the layer of mucus created and maintained by the goblet cells of the gut mucosa

Each community is distinct, even within the same compartment of the same animal, although there is considerable overlap. The mucosal community is the key to maintaining and, when necessary, restoring the individual's unique microbiota. Here's why:

The luminal community is constantly being moved downstream with the ingesta/digesta, through the various GI compartments and thus through some strikingly different microbiomes, and is eventually eliminated in the faeces. It also has to contend with ingested microbes that may be foreign to the horse's gut. In addition, the 'lumenati' is vulnerable to sudden changes in substrate (e.g., abrupt dietary changes) and thus the chemical composition of the microbiome.

The mucosal community is much more stable because it is protected within the mucus layer overlying the gut mucosa. It also benefits most from host interactions, including immune tolerance and defence against pathogens. However, the mucosal community is the one we know the least about in horses, as most studies use faeces as a proxy for the hindgut contents.

Note that the mucosal community is not the same as a biofilm. Biofilms are polymicrobial communities within an extracellular polymeric complex that is created and maintained by the microbes; and many of the microbes within this protective environment are in a hypobiotic state. In contrast, the mucosal microbial community in the gut exists within the mucus layer that is created and maintained by the host (e.g., the horse). In this context, biofilms are pathological; mucosal microbial communities are physiological.

One of the first gene-sequencing studies to map the microbiota of the healthy equine gut by compartment also compared the luminal and mucosal populations.⁹ There were three important findings:

1. There is a distinct difference between upper (stomach, small intestine) and lower (caecum, large colon) portions of the GI tract, greater in the luminal community than in the mucosal.
2. Microbial composition is much more variable — between horses and compartments — in the upper GI tract, and is remarkably uniform in the hindgut.
3. There is greater continuity through the length of the GI tract in the mucosal community.

Figure 7 illustrates all three points at the phylum and species levels.

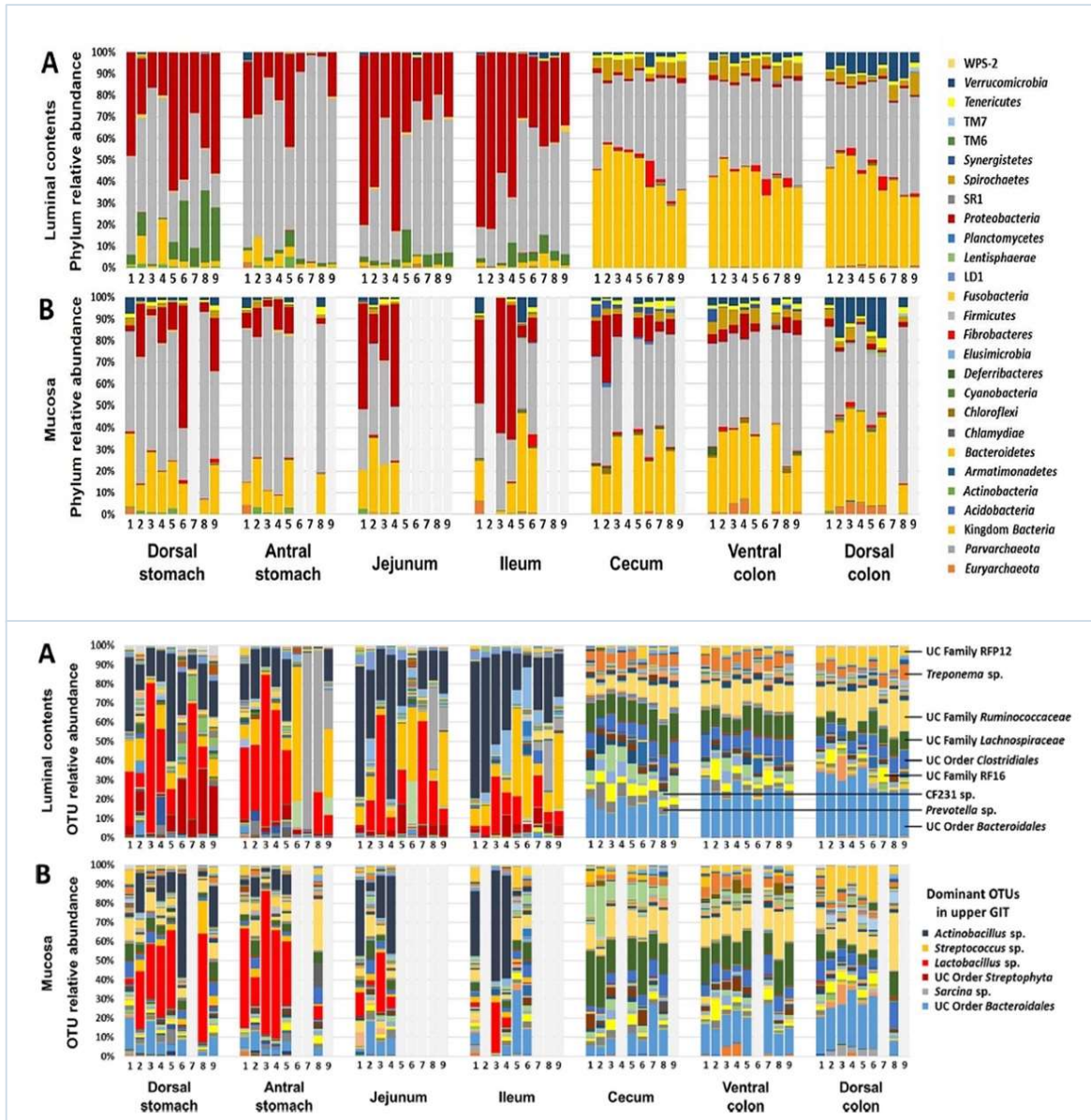


Figure 7. Relative abundance of the main bacterial phyla (top) and species (OTU; bottom), by compartment, in 9 horses without GI disease. **A**, luminal community. **B**, mucosal community. Dorsal and antral are the squamous and glandular portions of the stomach, respectively.⁹

5. The gut microbiota is established in the first 1–2 months of life

The gut microbiota in horses is established by 1–2 months of age, which is when coprophagy in foals is at its peak,¹⁸ grazing occupies at least 20% of the foal's day, and the caecum and large colon are rapidly increasing in size.¹⁹

Colonisation of the gut begins on day 1 with (primarily faecal) microbiota from the mare's vagina, perineum, flank, and udder as the neonate seeks the teat.^a In the first month of life, the microbiota is quite specific to this period, as the foal's diet is predominantly milk. The first month is also characterised by the lowest species richness and diversity of the entire developmental period.^{20,21}

With increasing amounts of solid food intake, the microbiota increasingly resembles that of an adult, each foal's microbiota being most similar to its dam's than to any other horse. (Not coincidentally, foals predominantly eat their own dam's manure during the coprophagy phase.¹⁸) After about 2 months of age, the foal's gut microbiota becomes relatively stable, resisting much change even through weaning (Fig. 8).²⁰

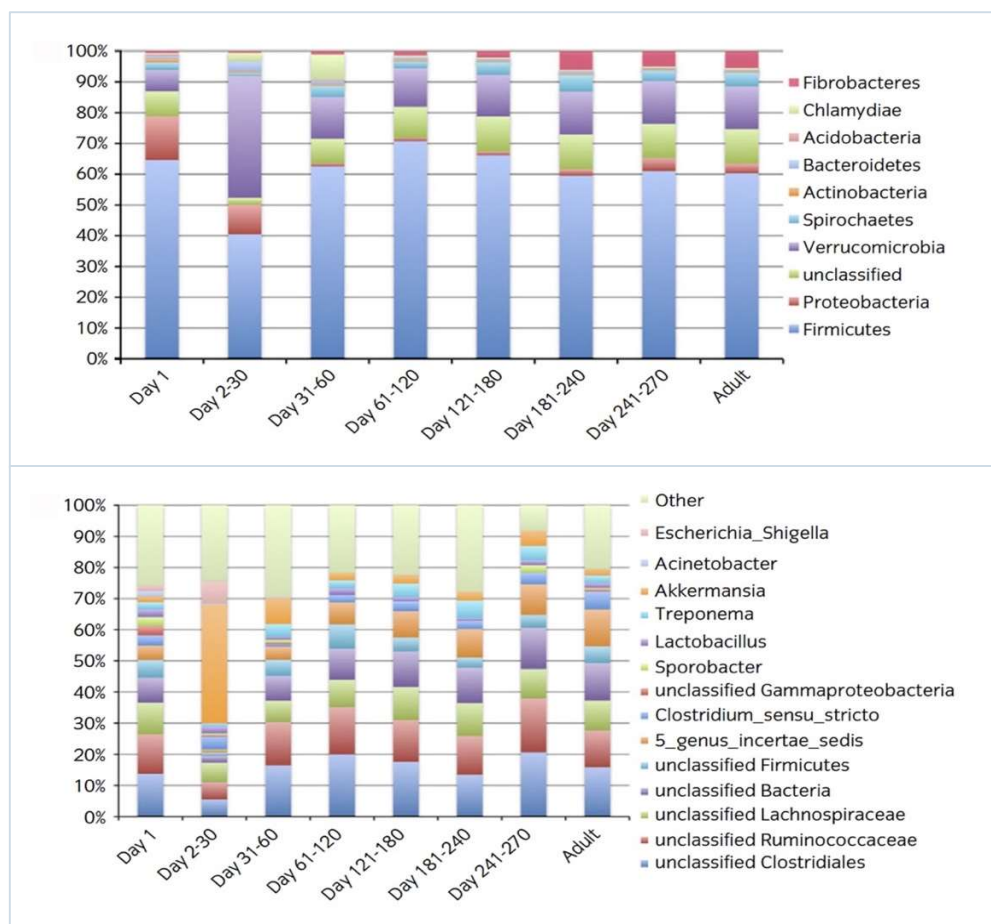


Figure 8. Relative abundance of the main bacterial phyla (top) and genera (bottom) in the faeces of 11 foals and their mares (Adult) from birth until 9 months of age. The foals were weaned at 6 months (~ 180 days).²⁰

Consistent with health outcomes in other species, a longitudinal study of Thoroughbred racehorses from birth to 3 years of age confirmed that bacterial diversity in the first 1–2 months of life, and particularly within the first 28 days, influences subsequent disease risk and athletic performance. Specifically, the incidence of respiratory, soft tissue, and orthopaedic disease/injury were each significantly higher, and racing performance and earnings were significantly lower, in foals with low faecal bacterial diversity in early life.²¹

6. Diversity characterises the healthy equine gut

In the context of the gut microbiota, diversity is an expression of both its richness (number of different species) and evenness (relative abundance of the various species). All else being equal, a more diverse community — *i.e.*, greater number of species, none especially dominant — is a more resilient one.

As we saw in the Welsh pony study (Fig. 5), a diverse community is not necessarily a stable one. In fact, a feature of complex, multispecies communities is that they are inherently vulnerable to instability.²² However, the potential for instability is countered by the stabilising effect of competition among species occupying the same niche. While that may seem counterintuitive, it is explained by the fact that co-operation or dependence among species renders them vulnerable to knock-on effects when a key species or group is lost. Thus, a diverse microbial community is resilient by virtue of its ability to change without becoming critically unstable.

Microbial diversity is an important feature of the healthy equine gut. Although specifics differ among studies, it is generally the case that healthy and well-performing horses have greater microbial diversity than their ill or poor-performing counterparts.^{16,21,23-26} The most-studied conditions include these:

- colic (acute and chronic)
- colitis or diarrhoea*
- equine gastric ulcer syndrome (EGUS)
- laminitis, particularly carbohydrate overload and endocrinopathic (hyperinsulinaemic) laminitis
- equine metabolic syndrome or insulin dysregulation (EMS / ID)
- obesity (*e.g.*, during weight loss management)
- behavioural reactivity (nervousness, 'flightiness', *etc.*)

* Surprisingly, in four separate studies, horses with free faecal water (excess of faecal liquor) did not show any change in microbial diversity compared with control horses (normal faecal consistency).^{23,24}

The following factors are also known to affect microbial diversity in the equine gut:^{1,6,11,12,16,20,21,23-32}

- dietary stress (*e.g.*, abrupt changes, high-starch diets, inadequate fibre, excessively mature fibre)
- antibiotic treatment, general anaesthesia, other drugs (*e.g.*, NSAIDs such as phenylbutazone)
- internal parasites, anthelmintic treatment
- exercise, transport, and other factors related to athletic training
- pregnancy (with gender as a proxy in feral horse studies)
- age or life stage, from foalhood to geriatric horse^b
- breed, particularly native/ancient breeds vs modern hybrids
- social factors such as hierarchy position and group interactions
- geographic location and local climate
- habitat and management, particularly confined vs free-range
- season, particularly in free-ranging horses and those on pasture

Glyphosate contamination probably belongs here, too, but it has not yet been studied in relation to the gut microbiota in horses.^c

Figure 9 provides an overview of some of the factors that can affect the gut microbiota, and the specific changes in important taxa that may be involved. But note that this diagram, while useful, is far from comprehensive. The factors that can affect the horse's gut microbiota, for better or for ill, could fill a book. The papers cited in this review are a tiny sampling of the veritable mountain of published literature to date.

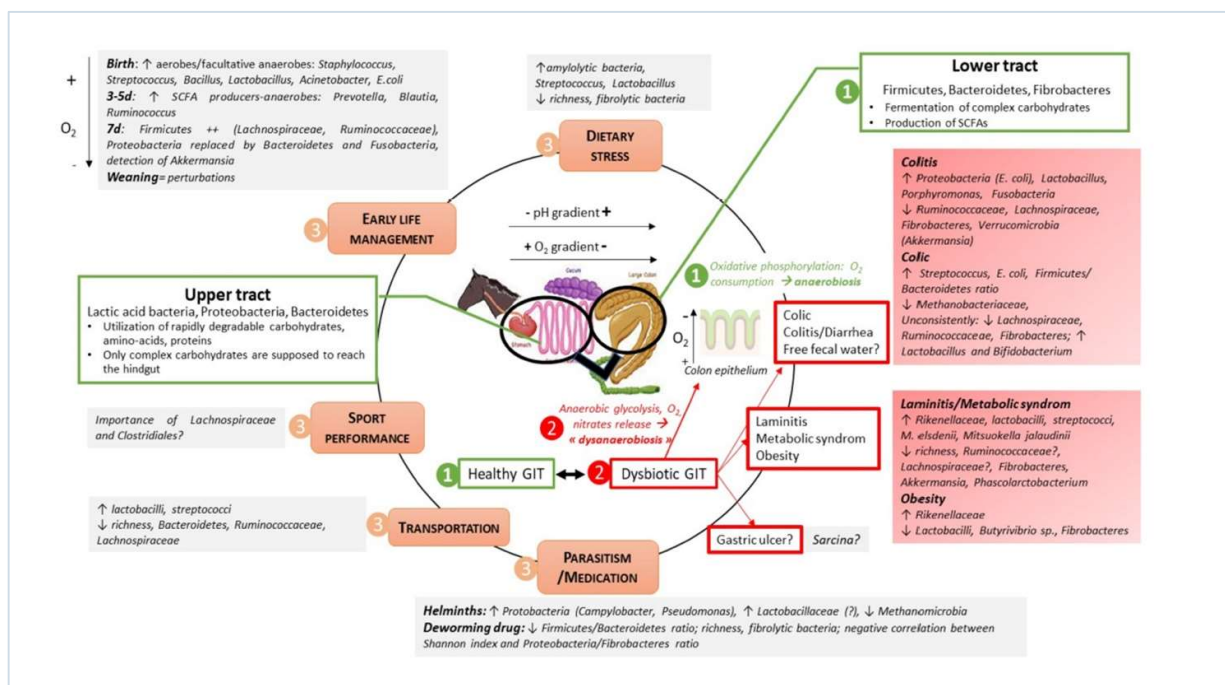


Figure 9. Overview of some of the main factors and microbial community members currently understood to contribute to gut health (in green) or dysbiosis (in red) in horses.²³

7. Microbial diversity is facilitated by a natural diet

When it comes to the gut microbiota, “who lives there?” is determined in large part by what there is to eat — by the substrates available to the microbes for their own nutrition, and by the resulting chemical composition of the microbiome. A diverse diet thus contributes to a diverse gut microbiota. The converse is also true: a compositionally limited diet leads to a less diverse gut microbiota.¹

Few studies have examined the differences in gut microbiota between domestic horses on pasture and those on a hay-based diet. The vast majority of studies distinguished only between high-starch and high-fibre diets, with pasture and hay diets lumped together as “forage” or high-fibre diets.

The studies that have deliberately or inadvertently compared the gut or faecal microbiota in pastured horses with those on hay-based diets have typically shown that horses on pasture have a more rich and diverse gut microbiota — from stomach to colon — than those on hay-based diets.^{8,29,30} However, it depends somewhat on the pasture (e.g., native vs cultivated; cool- vs warm-season grasses), the hay, and the time of year, particularly on the nonstructural carbohydrate (NSC) and crude protein content of the forages.³⁰

But what we do have is a handful of studies comparing the faecal microbiota of domestic and feral horses, ‘feral’ meaning once-domesticated breeds that have been free-ranging or free-living for generations, such as the Carneddau ponies¹² mentioned earlier and the Shackleford ponies of the Outer Banks (the barrier islands) of coastal North Carolina.³¹

Not surprisingly, feral horses generally have a more rich and diverse faecal microbiota than domestic horses (Fig. 10).^{1,31} In addition, the microbiota of feral horses appears better able to degrade complex carbohydrates such as cellulose¹ and create a more beneficial profile of volatile fatty acids in the presence of starch.³¹

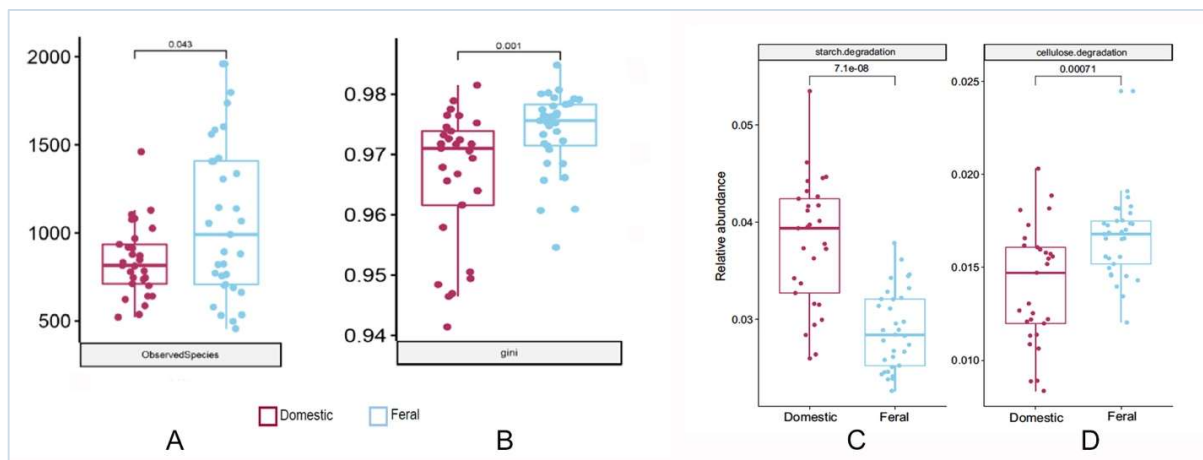


Figure 10. Differences in observed species (A), Gini diversity index (B), and the relative abundance of microbial genes coding for enzymes involved in starch (C) and cellulose (D) degradation, between domestic and feral horses in 5 countries.¹ Box plots show the median, middle 75%, and range (excluding outliers). Statistical significance between domestic and feral horses ranged from $P < 0.05$ (A) to $P \leq 0.001$ (B, C, D).

Regardless of location, the horse's natural diet comprises an enormous variety of plants and plant parts that changes in composition and nutrient profile with the seasons. Depending on the habitat (desert, mountain, plain, coast) and the time of year, free-ranging horses eat many different types of plants: mostly grasses, but also legumes and various other meadow and woodland herbs, shrubs, trees, and even mosses and lichens. In addition, the plant parts consumed (root, stem, bark, twig, leaf, flower, fruit, seed) varies with the season.

For example, in a year-long study of feral horses in the foothills of the Rocky Mountains in western Alberta, Canada in the 1970s, 43 plant categories (species or genus) were identifiable in the faeces over the course of the year.³³ In any given month, the diet included 17–21 different plant groups. Grasses, sedges, and rushes predominated, comprising 93% of the annual diet, and ranging from 88% in the late winter–early spring to 99% in the summer. Grasses alone comprised 56% of the annual diet (range, 48% to 70%). Overall, the identifiable genera included 17 grasses, 4 sedges and rushes, 8 forbs, 5 browse, and 3 'other' (horsetail, lichen, moss).

Even more remarkable, a review of 10 studies of wild burros (donkeys) living in the Mojave Desert in the southwestern United States found that 175 different plant species were consumed by burros in this desert landscape.³⁴ The faeces or stomach contents contained an average of 33 plant species *per* burro; it ranged from 11 to 54 species, depending on location and time of year.

Perhaps most astonishing yet is a new study of the Carneddau ponies in northern Wales. This particular study used plant DNA to identify individual plants in the faeces over a 2½-month period in the spring. In this cool, wet, wilderness environment, a total of 39 different plant families were identified in the ponies' faeces.³⁵ That's families, not genera or species. Those 39 plant families would potentially represent hundreds of different plant species, which is of a piece with the burro study, albeit in a much more generous landscape than the Mojave Desert.

In the Carneddau ponies, the predominant plant family was the grasses, which averaged 38% across the study (Fig. 11). Next most abundant were small shrubs (e.g., heather, bilberry) and rushes, averaging around 10% each over the season. But note how much variation there is in diet composition week-to-week!

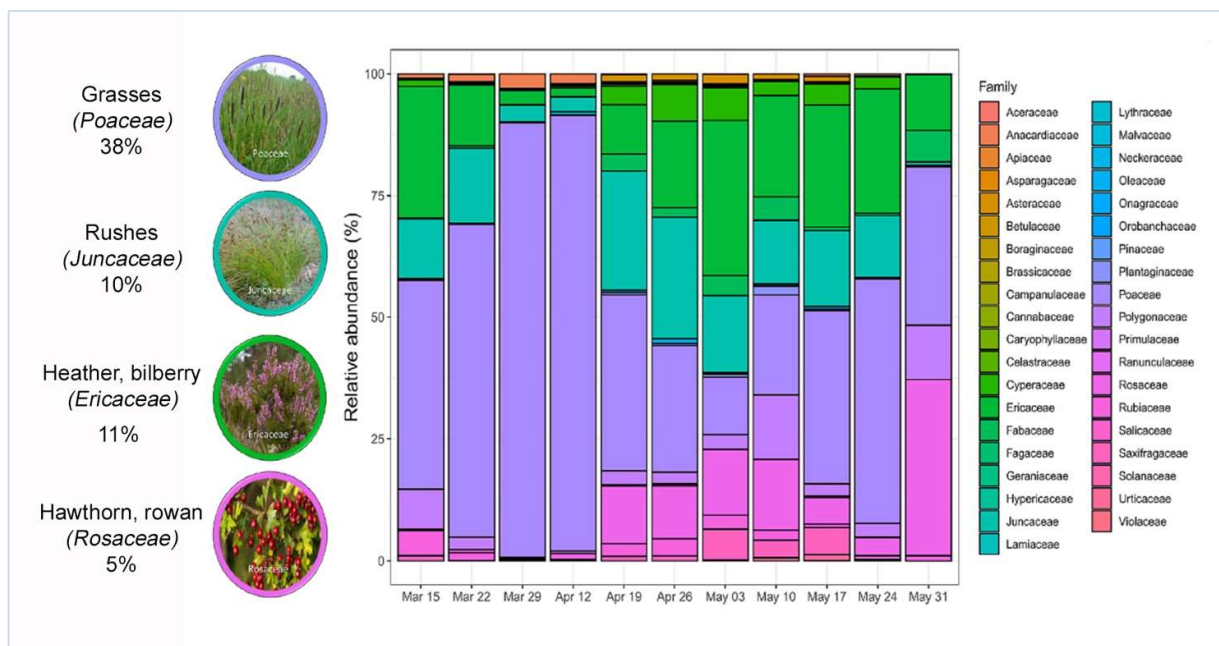


Figure 11. Weekly diet composition (relative abundance of the dominant plant families) of free-living Welsh Mountain ponies in the Carneddau mountains of northern Wales. Plants were identified by their DNA in representative faecal samples collected weekly during the spring.³⁵

Now consider how many plant families the average domestic horse eats, especially those on a hay-based diet, with little or no access to living plants (pasture turnout, hand-grazing, or hand-feeding). The average grass or cereal hay is a monoculture — a single species (e.g., rhodes, teff, timothy, barley, oat). A true meadow or mixed-grass hay is hard to come by for many horse-keepers.

The average “improved” pasture is not much better, typically containing a predominance of human-selected and perhaps even human-engineered grass species or varieties (strains),^{28,30} and very little plant diversity (“weeds”). Native pastures generally have much more diversity, but because our native pastures in Australia are not well adapted to large, hooved animals such as horses and cattle, they are easily overstocked, which dramatically decreases both their quantity and quality, including plant diversity.

Granted, free-ranging horses not subject to human management, such as supplemental feeding, can eat only what is available to them. So, there is a certain “make do” component to the horse’s natural diet. We must also consider the effects of exercise and other intrinsic and extrinsic factors listed earlier on the microbiota of free-ranging horses.

However, it’s not for nothing that the authors of the multinational study illustrated in Figs 1, 3, and 10 reached this conclusion:

“Our data showed an impoverishment of the fecal microbiome in domestic horses with diet, antibiotic exposure and hygiene being likely drivers.”¹

Impoverishment. What a perfect word to describe the average domestic horse’s diet and gut microbiota.

8. Feeding a variety of hays and herbs adds diversity to a limited diet

The horse's natural diet can also be characterised as being high in fibre and low in starch — but it is not always low in other NSCs, notably simple sugars and fructans (see Appendix). This entire class of plant carbohydrates is rapidly fermented by the gut microbiota, whether or not specific NSCs can be hydrolysed by the horse's digestive enzymes and/or cleaved by gastric acid.³⁶ In fact, there is always a race between enzymatic digestion and microbial fermentation of the hydrolysable carbohydrates, and it begins in the mouth.

During periods of rapid plant growth, which occurs whenever sufficient water, heat, and sunlight are available, the NSC content of pasture grasses and other herbage can exceed 30% of dry matter (DM).^{37,38} These periods pose a considerable digestive and/or metabolic risk for some horses and ponies, as the incidence of colic, diarrhoea, obesity, and laminitis all increase at these times.

When grazing a diverse pasture is unavailable or inadvisable, feeding as many different varieties of hay, chaff, or forage pellets as possible, considering the horse's nutritional and medical needs, is a good start. In keeping with the horse's natural diet, grasses should predominate, with some legume (lucerne, cowpea, burgundy bean, *etc.*) included for horses needing additional protein or variety. In at-risk horses and ponies, avoid cereal hays and chaffs, as they can have a water-soluble carbohydrate (WSC) content of > 40% DM.³⁸

Feeding fresh or dried herbs further increases the variety in a compositionally limited diet. Many of the medicinal herbs can also be fed for their nutritive value. In terms of quantity, herbs contribute little to the macronutrient profile of the horse's diet. However, they add a variety of micronutrients to the diet, and they provide the gut microbiota with an additional range of substrates — which may well be the same thing: feeding the microbiota feeds the horse.

The herbs best suited for this purpose are those that meet the following criteria:³⁹

1. Palatable to horses — plants that horses have been observed to eat in a natural setting (as part of their natural diet or when self-medicating) or that horses willingly eat when offered free-choice.
2. Safe for horses — herbs known to be safe for horses to eat in unlimited quantity, even though the horse may choose to limit intake.

Other considerations that are ideal but secondary include these:

3. Source — choose organically grown or responsibly wild-crafted herbs whenever possible, and buy from a company with ethical practices and high quality-control standards.
4. Plant conservation — avoid using wild-crafted plants on the United Plant Savers lists of critical and at-risk plants. (Not likely to be a big problem with herbs used for nutritive/diversity purposes.)
5. Breed origin — prioritise plants that are native to the region in which the horse breed originated; mainly Great Britain, western Europe, and the Middle East for Australian horses and ponies.
6. Seasonal variation — select herbs (plant type and plant part) for seasonal variety.



Figure 12. Close-up of a nutritive blend of dried herbs, formulated to add seasonal variety to a limited diet (see Table 2, next page, for the ingredients).³⁹

Offering the herbs free-choice is the best approach, as it allows the horse to select what, when, and how much of each herb to consume, based on current need and preference. But that is not always possible for horse-keepers. Blending dried herbs and storing the bulk mix for later use is more practical for most people.

When possible, create at least two seasonal blends that aim to mimic seasonal changes in plant types and plant parts. For example, Table 2 lists the ingredients of a primarily nutritive blend that was formulated for horses in North America. It comprises plants that are native or naturalised to that continent and thus to local breeds, and it emphasises the plant types and plant parts most abundant during the spring and summer.

Note that this blend emphasises the plant parts that are available to herbivores during most of the year: stems, leaves, flowers, and fruits (Fig. 12). The corresponding autumn-winter blend contains more roots, fruits, and seeds. This blend is presented simply as a starting point, as inspiration. Substitutions for individual herbs are not only possible, they are positively encouraged!

Table 2. The seasonal blend of nutritive herbs shown in Figure 12.³⁹

Ingredients	Parts*	Notes
Cleavers (<i>Galium aparine</i>)	1	Can be replaced with chickweed (<i>Stellaria media</i>)
Dandelion leaf (<i>Taraxacum officinale</i>)	1	
Dandelion root (<i>Taraxacum officinale</i>)	1	
Elder berries (<i>Sambucus nigra</i> , other <i>Sambucus</i> spp.)	1	Can be replaced with other berries, such as blueberry or bilberry (<i>Vaccinium</i> spp.)
Hawthorn leaf and flower (<i>Crataegus</i> spp.)	1	
Marshmallow root (<i>Althaea officinalis</i>)	2	
Nettle leaf (<i>Urtica dioica</i>)	2	
Raspberry leaf (<i>Rubus idaeus</i>)	1	
Red clover leaf and flower (<i>Trifolium pratense</i>)	1	Omit if fresh clover is available to the horse
Rose hips (<i>Rosa canina</i>)	1	

* For example, 1 part cleavers, 2 parts nettle leaf, etc., by weight.

Description: This seasonal blend is formulated to provide some of the meadow and woodland plants available to horses during the warmer months of the year. It is designed to expand the variety of plant-source nutrients in the horse's diet, using plants or plant parts that are naturally available during the growing season.

Instructions: Measure out all herbs and mix together well. (Note: dried raspberry leaf tends to clump, so break up any clumps as you mix.) Store the blend in a dry, airtight container, away from direct light and excessive heat. It should keep well under these conditions for up to 12 months, although it is best used within 6 months.

Suggested use: Offer free-choice daily from early spring to late autumn. If adding to the horse's food, give ½ to 1 cupful (1 to 2 oz.) per day for the average-size horse. Transition to the autumn-winter blend in late autumn.

(I typically recommend feeding the autumn-winter blend for only about 3 months each year, straddling the winter solstice, December 21-22 in the northern hemisphere. Transition to the autumn-winter blend in early November, and then back to the spring-summer blend in late January. Make the transition gradually, over a week or two.)

Alternatively, offer some or all of these herbs individually, and allow the horse to choose what and how much to eat each day. Dried herbs may be rehydrated before feeding, but discard any uneaten portion after 24 hours once water has been added. [Continued on the next page.]

Notes: The herbs in this blend are all safe to feed in larger amounts than those suggested here. The daily amount recommended to feed is intended to be a balance of effectiveness and economy. You can safely feed more than 2 oz/day if you desire.

Footnotes

^a Establishment of the foal's gut microbiota may actually begin *in utero*, as the uterus, and thus the placenta, amniotic fluid, and meconium each have their own microbiota.^{40,41} And then there is the seminal microbiota, which no doubt contributes to the uterine and foetal microbiota.⁴² These microbial communities are far more limited than the gut microbiota, but the fact remains that life does not begin in a sterile environment.

^b In general, microbial diversity (particularly species richness) declines with age. In one recent study of horses aged 6–30 years, the divergence occurred at 16–20 years of age.⁴³ However, not all senior horses had decreased diversity; ~ 55% of horses aged 21–30 years had species richness comparable to younger horses. Overall, only ~ 50% of the variability in species richness among horses was attributable to horse age.

^c According to its US patent, glyphosate is an ultra-broad-spectrum antimicrobial agent.⁴⁴ The lack of consistent *in vitro* effects on the ruminal microbiota of cattle is at odds with clinical reports of adverse effects on health, production, and fertility in cattle and other livestock species consuming glyphosate in feed. Its effects on the microbiota of the equine gut have not yet been studied. However, a German study found glyphosate in the duodenal fluid and blood of all horses undergoing upper GI endoscopy for colic, weight loss, diarrhoea, anaemia, or poor performance.⁴⁵ Median duodenal concentrations in horses with severe gastric ulceration were > 2X higher than in horses with normal gastric mucosa.

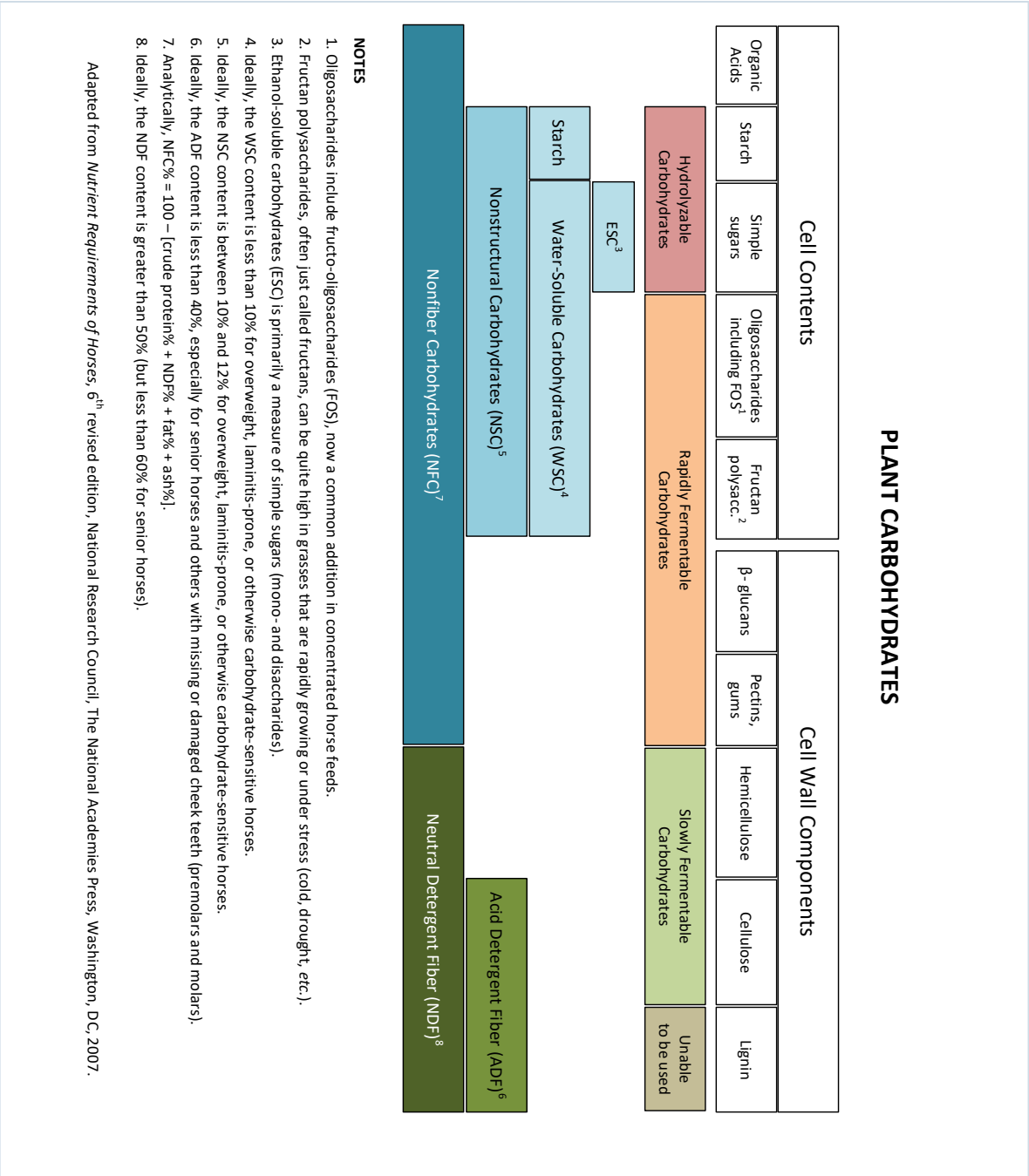
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Appendix. Specific types of plant carbohydrate considered in equine nutrition.



NOTES