

Intestinal Microbiota and Health The Gut and Beyond

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The gastrointestinal (GI) tract is a complex organ that has important total body health functions that extend far beyond the simple orchestration of nutrient absorption. The unique effect that the GI tract has on systemic health rests within the enteric microbial population that resides along its length. Enteric microbes guide the maturation of the immune system and the immune response to perceived antigens. These microbes work by modulating systemic inflammation, affecting neurologic development and function, and by influencing other metabolic processes. Dysbiosis of the gut microbiota is associated with gastrointestinal disorders as well as systemic perturbations that affect the health of horses.

Areas of Support Provided by a Healthy Gut Microbial Population

Gastro-Intestinal Disorders

Research into the influence of gut bacteria on diarrhea, ulcerative colitis, and colic is growing. Foals that develop diarrhea by the age of 28 days have a different gut microbial makeup compared to healthy foals.¹ Specifically there is an underrepresentation of the families *Lachnospiraceae* and *Ruminococcaceae* in diarrheic foals. Adult horses with diarrhea have decreased microbial diversity, both in richness (how many different species are identified) and evenness (how well represented are all of the species).² Antibiotic treatment in horses, which can wipe out beneficial as well as pathogenic gut microbes has been shown to increase the prevalence of *Salmonella spp.* and *Clostridium difficile*.³ Intestinal adsorbants, like DTO-smectite, that bind to pathogenic toxins, can decrease the incidence of post-operative diarrhea,⁴ suggesting a role of gut dysbiosis and its toxigenic effect on diarrhea.

The intestinal microbiota can also have a profound influence on colitis. The structure and composition of the gut microbiota in mice with severe colitis, as compared to mice with milder colon damage, have

unique features, such as an increase in *Akkermansia* bacteria and a decrease in *Barnesiella spp.*⁵ In humans, it has been noted that alpha diversity (how many species exist and how well-represented they are) is decreased with ulcerative colitis.⁶ Dysbiosis, or perturbation in the healthy microbial population, is noted in horses with colitis, as well. For example, undifferentiated colitis in horses is associated with less *Firmicutes* but more *Bacteroidetes* and *Proteobacteria* than healthy horses.⁷

The gut microbiota plays a role in the development of colic. Abrupt dietary changes may induce dysbiosis and create an environment favorable to the overgrowth of *Clostridium botulinum* type C.⁸ Researchers have found an abundance of the *Clostridium botulinum* toxin among horses with colic associated with acute equine grass sickness.⁹ Weese and colleagues reported horses that develop post-partum colic have an unusually low abundance of *Firmicutes* and an overgrowth of *Proteobacter*.¹⁰ In fact, 100% of horses with a relative abundance of *Firmicutes* below 50% (n = 8) developed post-partum colic.

Drug-Induced Dysbiosis

Administration of certain drugs can induce gastro-

intestinal dysbiosis in horses. Non-selective antibiotics can destroy populations of bacteria, pathogenic and beneficial. In horses, certain antibiotics disrupt the normal intestinal tract microflora, notably through a decrease in alpha-diversity and a particularly significant decrease in the *Verucomicrobia 5 genus incertae sedis*.¹¹ This bacterial species is abundant in the large colon and feces of healthy horses.¹² In addition to damaging the gastric lining, non-steroidal anti-inflammatory drugs (NSAIDs) disrupt the lining of the small intestine.¹³ This damage is not acid-related and is now considered to be a factor of the small intestinal microbiome.¹³ Both non-selective NSAIDs and COX-2 inhibitor NSAIDs decrease microbial diversity in the feces of adult horses.¹⁴

Feed Efficiency

The gut microbiota plays a fundamental role in the ability to digest and extract energy from nutrients, a function termed feed efficiency. Over a dozen specific gene sequences or operational taxonomic units (OTUs) have been identified that are associated with improved feed efficiency in the pig.¹⁵ Lactic acid bacteria abundance has also been linked to high feed efficiency in pigs.¹⁶ In fact, probiotic administration to maintain the gut microbiome in pigs supports growth and performance with the same efficiency as antibiotics.¹⁷ In lambs, improved feed efficiency is measured in conjunction with increases in both rumen- and colon-located fibrolytic taxa and with reductions in *Proteobacteria* located in the small intestine and distal gastro-intestinal tract.¹⁸ Interestingly, cows with low bacterial richness have an improved feed efficiency over cows with greater richness.¹⁹ The effect of probiotics on equine feed efficiency have been evaluated in research studies and are discussed below.

Immune and Inflammation

It is estimated that approximately 70% of all immunologically active cells reside within the gut.²⁰ Furthermore the maturation of the immune system is microbiota-driven. Microbial colonization promotes effective innate and acquired mucosal immune systems, epithelial renewal, barrier integrity, and

mucosal vascularization and innervation.²¹ When probiotics are provided as part of a diet supplement, support of immune function is often noted.²² In germ-free rodent models, the inflammatory response to chemically-induced colitis is heightened when compared to counterparts with a healthy microbiota.²³ Gut dysbiosis very early in life can negatively impact the immune response, particularly the response to allergens, years later.^{23,24} High acetate-producing microbial species flourish on a high-fiber diet. Acetate stimulates an increase in numbers of circulating T regulatory white blood cells (*Treg*) and is associated with a decrease in the severity of asthma symptoms.²⁵ Even food allergies can be mitigated by a healthy gut microbiota.²⁶

Intestinal microbiota can also impact systemic, low-grade inflammation. A healthy, normal microbiota increases the anti-inflammatory compounds transforming growth factor (TGF) and interleukin (IL)-10.²⁷ This change in anti-inflammatory mediators subsequently increases circulating Tregs. Furthermore an ideal gut microbiota decreases pro-inflammatory mediators including tumor necrosis factor-alpha (TNF- α), IL-6, and IL-12.²⁷ Gut dysbiosis has also been implicated in systemic inflammatory disease. For example, distortions to the normal microbiome has been linked to rheumatoid arthritis.²⁸

Neurologic, Cognitive and Mood

Disruptions in the gut bacterial population alters intestinal permeability, which then can impair the integrity of the blood brain barrier. The end result is that the brain becomes more vulnerable to the influx of deleterious substances from the systemic circulation. Neuroinflammation and cognitive dysfunction then can develop.²⁹ Post-weanling mice that have been depleted of their gut microbiota show lower levels of the neurologically supportive compound brain-derived neurotrophic factor (BDNF).³⁰ BDNF controls many neurophysiological activities, specifically nerve cell development, neuroprotection, and modulation of synaptic interactions critical for cognition and memory.³¹ The gut microbiome can even influence behavioral traits because the traits can be transferred from one animal to another via the microbiome.³²

One very intriguing area of current research focuses on the impact of the gut microbial composition on neurological disorders, such as Alzheimer's Disease, Parkinson's Disease, and multiple sclerosis.³³⁻³⁵ Therapeutics for depression have also been suggested to address the gut microbial population.³⁶

Metabolic support

Certain systemic metabolic processes can be affected by the enteric microbial population and investigations into the gut microbiota's role in disorders like obesity and insulin resistance are growing. Fecal transfers from obese humans and mice to healthy, lean mice causes weight gain in the once-lean group.^{37,38} Low intestinal tract bacterial numbers and richness are noted in individuals with insulin resistance and dyslipidemia.³⁹ One suggested mechanism of metabolic support is through the actions of short chain fatty acids (SCFA), which are byproducts of the bacterial fermentation of non-digestible fibers. Butyrate, propionate, and acetate are all SCFAs which are produced with the bacterial fermentation of cellulose and hemicellulose in the gastrointestinal tract. Supplementation with butyrate appears to improve insulin signaling and prevent weight gain in rodents.⁴⁰ Acetate appears to increase glucose-stimulated insulin secretion in rodents.⁴¹ People with type 2 diabetes reportedly have a reduced number of SCFA-producing bacteria.⁴² In horses with metabolic syndrome, there is significant decrease in microbial diversity with *Fibrobacter*, a group of fiber-digesting bacteria, being particularly underrepresented.⁴³ Laminitis induction via starch infusion causes a shift in the equine microbiome with a notable increase in *Streptococcus* and two potentially-pathogenic, gram negative bacteria, *Veillonella spp.* and *Serratia spp.*,⁴⁴ suggesting a potential causal role of certain pathogens in the development and clinical symptoms of laminitis.

Bone density

Research relating the health of the gut microbiota to bone density is emerging. A few of the mechanisms include control over the production of inflammatory cytokines and subsequent regulation of osteoblast

and osteoclast activity,⁴⁵ production of steroids and serotonin, and calcium homeostasis.⁴⁵ Chronic antibiotic treatment has been shown to impair whole bone mechanical properties.⁴⁶ Intestinal overgrowth of pathogens impairs absorption of nutrients critical for bone health (e.g., calcium, carbohydrates, B vitamins, and vitamin K).⁴⁷ The increase in microbial by-products, specifically SCFAs, are suggested to improve fractional calcium absorption following probiotic supplementation⁴⁸ leading to more calcium availability for bone mineralization.

Modes of Support for Healthy Gut Microbiota and Beyond

Probiotics

A probiotic is a living micro-organism(s) that, when consumed in sufficient quantity, confers a health benefit to the host.⁴⁹ Probiotics can directly or indirectly, through their metabolites, modulate the immune system.⁵⁰ This may occur through the stimulation of intestinal epithelial cells (IECs) and gut-associated immune cells, which results in improvements in gut barrier function and immune defenses.^{50,51} Probiotics can increase Treg cell numbers,⁵² downregulating misdirected immune responses. Certain probiotics modulate inflammatory pathways, such as the pro-inflammatory products of NF-kappa beta and the anti-inflammatory products of PPARgamma,⁵¹ Increased intestinal IgA production and altered systemic IgG isotypes are other potential benefits conferred by probiotics on the immune system.⁵¹ Probiotics can inhibit pathogen attachment to mucosal cells thus inhibiting pathogen growth and colonization.⁵³ This effect is termed "competitive exclusion."⁵⁴ Furthermore, certain probiotics contain outer shell components that bind pathogens and further prevent their adherence to the mucosa and enhance pathogen removal.⁵⁵ A good body of literature supports the role of probiotics in bone health^{56,57} and metabolic disorders.⁵⁸

Prebiotics

Prebiotics are non-live compounds that are indigestible to the host and selectively improve the activity and numbers of beneficial microbes in the

gastrointestinal tract.⁵⁹ Prebiotics have demonstrated anti-inflammatory benefits in the foal, as noted by reductions in pro-inflammatory cytokines.⁶⁰ An oligosaccharide prebiotic has been reported to improve insulin sensitivity in obese horses.⁶¹ Similar results have been noted in obese dogs.⁶² Rodent models suggest that prebiotics can modulate memory and learning.⁶³ In humans, non-starch polysaccharide supplementation has been linked to improvements in recognition and working memory performance.⁶⁴ Prebiotic supplementation in piglets was shown to reduce anxiety-related behavior.⁶⁵ Noted changes in mood/behavior are suggested to be due to structural changes as prebiotic supplementation in piglets has been associated with reduced gray matter, suggesting an improvement in neural pruning.⁶⁵ Prebiotics are well-documented as having immune-modulating effects and can augment the adaptive immune response to vaccinations and pathogens.⁶⁶ Similar to probiotics, mineral absorption, specifically calcium and magnesium, can be enhanced with prebiotic supplementation.⁶⁶

Metabolites

Butyrate is an SCFA produced by microbes following fermentation of certain polysaccharides. One of the benefits attributed to a healthy gastro-intestinal microbial population is the production of butyrate, for this particular metabolite has immune-enhancing and metabolic-improving effects as well as enterocyte-supporting benefits. Research in rodents report that butyrate supplementation attenuates intestinal inflammation in a colitis model.⁶⁷ A benefit possibly due to butyrate's inhibitory effect on NF-kappa beta.⁶⁸ In ponies, supplementation with the various SCFAs showed that butyrate, alone, had insulin-sensitizing effects.⁶⁹ The provision of butyrate to the diets of mice has resulted in improvements in insulin function.⁷⁰ Butyrate has been suggested as a possible alternative to antibiotics as a means of improving host innate immunity.⁷¹ Indole is a microbe-derived metabolite of tryptophan. Indole improves gut barrier function and intestinal inflammation.⁷² Indole has been shown to reduce the severity of infection with *Clostridium difficile* in mice.⁷³ Dietary indole improves

formation of Tregs and moderates formation of Th17 cells, suppressing delayed-type hypersensitivity.⁷⁴ Lastly, indole supplementation can prevent NSAID-induced dysbiosis of the gut microbiota.⁷⁵

Omega-3 fatty acids

Animals deficient in omega-3 fatty acids show intestinal dysbiosis with a decrease in the number of *Bacteroidetes* and *Firmicutes* in aged mice.⁷⁶ Omega-3 fatty acid supplementation prevents the overgrowth of the potentially pathogenic *Enterobacteriaceae*.⁷⁶ Human research suggests omega-3 fatty acids can alter the microbiome such that there is an increase in SCFA-producing micro-organisms.⁷⁷ With the wealth of knowledge on the benefits of omega-3 fatty acids for health and disease prevention, it is possible one route to omega-3 supported immune, inflammatory, and metabolic functions is through gut microbiota modulation.

Antioxidants

Supplemental antioxidants have been shown to support gut health and healthy microbial populations. For example, piglets supplemented with an antioxidant juice showed improvements in antioxidant status as well as increases in facultative probiotic bacteria and lactic acid bacteria.⁷⁸ Another swine study demonstrated that antioxidant polyphenol supplements can decrease the expression of pro-inflammatory cytokines as well as the growth of pathogenic microbes (e.g., *Streptococcus* and *Clostridium Cluster XIV*).⁷⁹ Some antioxidants exert their beneficial effects on gut microbiota via their antimicrobial activities.⁸⁰ Fiber carries certain polyphenolic antioxidants through the intestinal tract. At the colon, microbial activity releases the fiber and the polyphenol creates an antioxidant environment.⁸⁰

Lifestyle

Both diet and exercise can have a profound effect on the gut microbiota and, thus, on various aspects of equine health. The equine diet of predominantly forage with low levels of non-structural carbohydrates supports a healthy gastro-intestinal microflora. When comparing a hay diet to a hay + oats diet, it was noted that the high nutrient availability diet (hay + oats) was associated with a lower microbial

diversity.⁸¹ Differences have also been reported when comparing horses on a pasture-only diet to those on a concentrate diet.⁸² For example, the concentrate group showed a greater population abundance of the *Bacillus-Lactobacillus-Streptococci* group, which are lactic acid-producing species that prefer hydrolysable carbohydrates, although they do not ferment these carbohydrates directly. Despite the diet-induced increase in colonic lactate, there was no concomitant increase in the lactate-utilizing group *Veillonellaceae*. This study also reported that Fibrobacter, which are fibrolytic bacteria, are significantly reduced on a high concentrate diet. A high-starch diet can be linked to an increase in amylolytic bacteria as well as stress-associated behavior.⁸³

It is now recognized that exercise training induces adaptations in the gut microbiome, and these changes parallel health benefits of exercise, such as improvements in metabolism, immunity, and behavior.^{84,85} Furthermore, exercise-induced gut microbial environments are being associated with freedom from disease, such as tolerance to high-fat diet, experimental diabetes, and toxin-induced dysbiosis.^{84,86,87} Exercise has been associated with an increased production of butyrate.⁸⁸ Fecal microbial transplant studies in rodents demonstrate that the microbiota of exercised mice could confer intestinal health benefits to previously germ-free mice.⁸⁹ In addition, transplanting the fecal microbiota of moderately exercised mice to germ-free mice conferred a significant resistance to induced colitis.⁹⁰

Summary

In summary, the gastro-intestinal tract guides much more than just nutrient absorption. It is linked to immune and inflammatory conditions, metabolic disorders, bone and cognitive health, as well as other conditions. Support for the gastro-intestinal microbes can come in the form of various types of supplements, such as probiotics or their metabolites, prebiotics, micronutrients, and fatty acids. Furthermore, a healthy lifestyle consisting of a high-fiber, forage-based diet low in concentrates and non-structural carbohydrates and exercise can further support a healthy gut microbial population.

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