Research: The Impact of Digestive Shield™ on Digestive Health, Immune Health, and Inflammation

Digestive Shield™ is a proprietary dietary blend of pre-, pro-, and postbiotics, marine calcite, and controlled starch to support a horse's gut and immune health. Each of these components can individually support gut and/or immune health, and when combined, these ingredients work synergistically to promote gut and immune health as well as reduce whole body inflammation in healthy horses. Each component was selected based on their actions on all or part of the equine gastrointestinal tract. Marine calcite acts primarily to buffer stomach acid while controlled starch can be helpful in the stomach and intestines. Prebiotics like inulin, a fructooligosaccharide found in chicory root, are preferentially fermented in the hindgut by beneficial bacteria while multi-strain probiotics, including *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium thermophilum, Enterococcus faecium, and Bacillus subtilis*, work throughout the intestines to support microbial diversity and stabilize the gut microbiome. Finally, a postbiotic, specifically a yeast culture product from *Saccharomyces cerevisiae* fermentation, can contain metabolites that are beneficial throughout the gastrointestinal tract and stimulate an appropriate immune response.

Impact of Prebiotics on Equine Health

Prebiotics are types of fibers that beneficial bacteria in the digestive tract preferentially ferment. Fructooligosaccharides (FOS), including inulin, are a type of prebiotic that have shown various beneficial effects on equine health, particularly in terms of gut health and immune functions.

FOS supplementation helps stabilize the intestinal microflora in horses, especially during dietary changes. In one study, when horses were abruptly introduced to barley, FOS supplementation prevented significant increases in *Lactobacillus* and *Streptococcus* populations, which were observed in the control group without FOS [1], indicating that FOS can mitigate microbial disturbances in the hindgut. Additionally, FOS can buffer the effects of starch overload, thereby reducing the risk of digestive disturbances by maintaining this stable microbial environment and pH balance in the hindgut [1, 2].

The preferential fermentation of FOS by beneficial hindgut microbes leads to increased production of volatile fatty acids (VFAs) such as acetate, propionate, and butyrate which fuel the colonic cells, supporting cell health and function and maintaining gut integrity. The increased production of VFAs resulting from FOS supplementation can decrease fecal pH [2, 3], decreasing potentially harmful bacterial populations like *Escherichia coli* [2]. These changes in VFA production and fecal pH in horses fed prebiotics can contribute to improving fiber digestibility [4], indicating an improvement in gut function since digestibility is an indicator of function [5-8].

In addition to promoting digestive health, FOS may also play a role in modulating the immune system. Supplementation has been associated with increased levels of immunoglobulin M (IgM) in blood [3] and immunoglobulin A (IgA) in the intestines [9], indicating an enhanced immune response and readiness.

These studies support different beneficial effects of adding FOS to equine diets on digestive and immune health and demonstrate the reason FOS was selected to be a part of Digestive Shield™.

Impact of Probiotics on Equine Health

Microorganisms live symbiotically in the horse's digestive system. Mostly concentrated in the hindgut (cecum and large intestine), these microorganisms work to break down the fiber in feed and hay. Gut microorganisms include bacteria, protozoa, fungi, and even viruses. The specific combination of organisms acts as a unique ecosystem within the horse. When the populations of microorganisms in the gut are balanced, the horse can achieve maximum nutrient digestion from its forage and feed. When they are unbalanced, the horse's immune, digestive, and other systems can be affected.

Probiotics are living microorganisms that can be added to a horse's diet that impart benefits on gastrointestinal and immune health. In general, probiotics help stabilize the microbiome, helping maintain cecal pH even when horses are fed high-concentrate diets [4]. Combined with prebiotics, probiotics like *E. faecium* can improve fiber digestibility [5]. Another combination of *L. casei, B. bifidum,* and *E. faecium* improved mineral and fat digestibility when fed to mature geldings [10], indicating a positive effect on overall digestion and intestinal health. Additionally, probiotic supplementation can enhance exercise performance by reducing post-exercise blood lactate levels, suggesting a switch in muscle energy source from carbohydrates to short-chain fatty acids [11].

Probiotics can help positively affect microbial diversity of the large intestine, reducing opportunity for pathogenic bacteria and supporting healthy immune responses. Research has shown a reduced microbial diversity within older horses [12], horses transported for long periods [13], and those fed high-starch diets [14]. These situations can be common in performance horses, so greater microbial diversity and better mucosal integrity may provide more stability and resistance to dietary changes, age, and stress. Supplementation with *L. acidophilus* can promote an increase in the relative abundance of the *Lactobacillus* genus and higher levels of beneficial short-chain fatty acids (SCFAs) like butyric and valeric acids [15]. This suggests a potential for improving gut health and stability without causing significant disruptions or acidification. In a randomized clinical trial, a mixture containing *L. acidophilus, L. casei,* and *E. faecium* reduced fecal *Salmonella* shedding in hospitalized horses [16], indicating that the probiotic positively influenced horses' microbiomes during a time of stress. Additionally, *L. acidophilus* combined with other probiotic strains can help maintain mucosal integrity and modulate local immune responses, especially during events which can disrupt the microbiome [17]. Horses fed *E. faecium* had increased plasma concentrations of IgM, indicating a greater immune readiness horses fed no probiotics [3]. These impacts

demonstrate that probiotics positively influence microbial diversity to support gastrointestinal health and immune response.

These studies illustrate the positive impacts of adding probiotics, such as *B. thermophilum* and *B. subtilis*, to equine diets and demonstrate the benefits of multi-strain probiotic mixtures that contain *L. acidophilus, L. casei,* and *E. faecium*. By including a multi-strain probiotic, Digestive Shield™ can positively impact digestive health as well as immune and inflammatory responses.

Impact of a Postbiotic (*Saccharomyces cervisiae* fermentation product) on Equine Health

When yeast and/or bacteria are fermented outside the animal they create metabolites that can have beneficial health effects. A postbiotic is the combination of inanimate microorganisms, both intact and cell well fragments/structures, and their metabolites that when fed back to an animal confer a health benefit. These metabolites when consumed in the feed as postbiotics then serve as signaling molecules for a variety of physiologic functions. Research on a particular postbiotic known as *Saccharomyces cerevisiae* fermentation products (SCFP) in horses has explored SCFP effects on digestive health, immune response, stress response, and inflammation.

SCFP can positively impact the horse's digestive tract by improving fiber digestibility and stabilizing the gut microbiota [8, 18]. After vaccination, SCFP supplementation can modulate early immune responses, affecting leukocyte subpopulations and enhancing IgG titres [19, 20]. Additionally, SCFP can reduce markers of inflammation in horses. Following an injection of an inflammatory compound, horses consuming SCFP had lower levels of prostaglandin E2 (PGE2), an inflammatory marker, and more favorable markers of collagen synthesis in their joints [21] Other inflammatory markers have also been lowered after inflammatory injection in horses fed SCFP [22]. Supplementation with SCFP can mitigate exercise-induced stress by reducing cortisol levels and inflammatory cytokines post-exercise [23]. Exercised yearling Quarter Horses fed SCFP had reduced serum cortisol, a stress biomarker, and serum amyloid A, an inflammatory biomarker, at 6 hours post-exercise compared to control horses [23].

The above research indicates that SCFP has beneficial effects on digestive health, immune, stress, and inflammatory responses. By including SCFP as part of the total package, Digestive Shield™ can promote these aspects of equine health.

Impact of Marine-sourced Calcite on Equine Health

As the horse eats food passes first into the stomach. The stomach is designed to serve as a mixing vat and begins the digestive process through the addition of strong acids to the food mixture. Because horses were designed to eat continually, horses produce stomach acid at all times, not just after meals. When horses are instead meal fed or go extended periods without food, this stomach acid on an empty stomach can be problematic. Normally, when grazing, horses produce a great deal of saliva that tends to buffer stomach acid. However, if grazing/chewing is reduced, saliva production is also reduced and stomach acid can get out of hand. When the gastric pH gets too acidic, conditions become prime for gastric ulcers.

Incorporating marine-sourced calcite derived from red algae into equine diets can help prevent gastric ulcers. Marine-sourced calcite has a unique structure which makes it highly digestible as well with a higher absorption than limestone, a traditional source of calcium [24]. Marine-derived calcite increases the pH of the equine stomach [25], thereby reducing the risk and severity of gastric ulceration [26]. When compared to traditional sources of calcium, it has over twice the buffering capacity [25]. In an internal study, horses fed a pellet containing marine-sourced calcite exhibited higher gastric juice pH levels at 2 and 4 hours post-feeding compared to baseline[27], indicating effective buffering of stomach acid.

In addition to helping buffer in the stomach, research has also shown a beneficial buffering effect of calcite in the horse's hindgut (cecum and large intestine)[28]. In times of extreme stress or illness, the horse's hindgut can experience a rapid decline in pH, known as acidosis. This acidic environment can be damaging to the microbial population, as well as cause inflammation in the gut itself and initiate a cascade of damaging effects for the horse. Helping to buffer, or maintain, hindgut pH can help keep the horse's hindgut functioning normally.

Impact of Starch on Equine Digestive Health

Starch longed been linked to digestive health in horses. Starch in the diet that escapes digestion in the small intestine can ferment rapidly in the hindgut and overwhelm the horse's ability to clear the VFAs produced [29], leading to hindgut acidosis and an increased risk of laminitis. High-starch diets alter hindgut microbial populations, shifting bacterial populations away from fiber fermenters [30-33], and increase risk for equine gastric ulcer syndrome (EGUS) [34-36]. Controlling starch and other non-structural carbohydrates to less than 25% of the feed lowers the risk of hindgut acidosis and can reduce gastric ulcer numbers and severity [35, 37]. Overall, while starch is a common component in equine diets, its high intake can lead to digestive health issues. As a part of Digestive Shield™, the controlled starch works with other ingredients as well as on its own to support digestive health in horses.

Research on Digestive Shield™ Package and Inflammation

Recently, research on Digestive Shield™ as a dietary package was published indicating that the proprietary blend of pre-, pro-, and postbiotics, marine calcite, and controlled starch influences inflammatory and immune pathways as well as oxidative stress. In these studies,

twenty 2-year-old horses were divided evenly into two groups (n=10 per group) to receive either a control diet (CON) or a diet with Digestive Shield™, and researchers were blinded to diet. One study [38] found that in healthy horses Digestive Shield™ reduced inflammatory mediator tumor necrosis factor (TNF)-α by 34% ($P < 0.05$) and tended to reduce inflammatory mediator interleukin (IL)-6 by 26% ($P < 0.10$) in blood following a submaximal exercise test. The same study found that other key immune mediators IL-8 and IL-18 were greater by 13% and 29% ($P < 0.01$), respectively, in Digestive Shield™-fed horses when compared to controls (Figure 1).

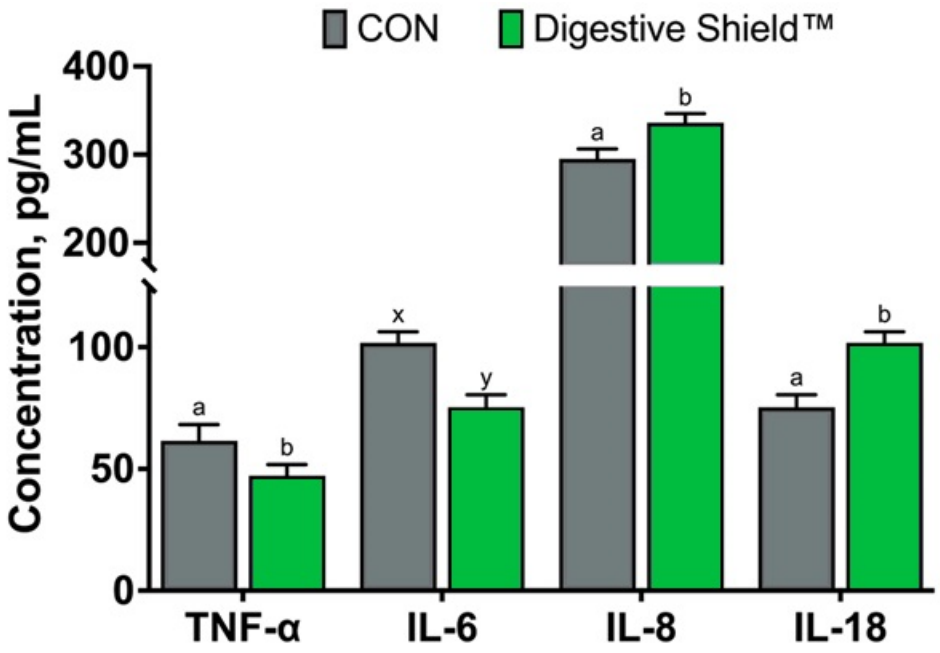


Figure 1. Whole-body inflammation markers in control (CON) and Digestive Shield™-fed horses following a submaximal exercise test [38].

Another study also determined the systemic inflammatory response in healthy horses fed Digestive Shield™ after an antibiotic challenge [39]. In this study, Digestive Shield™-fed horses again had lower inflammatory mediators TNF-α by 49% and IL-10 by 46% ($P < 0.05$ for both) in circulation than controls and did not lower any key immune messengers (Figure 2). TNF-α and IL-6 promote inflammatory processes throughout the body while IL-8 and IL-18 promote immune responses and activation. IL-10 is considered anti-inflammatory but is produced in response to inflammation, indicating that Digestive Shield™ horses had less inflammation in both studies.

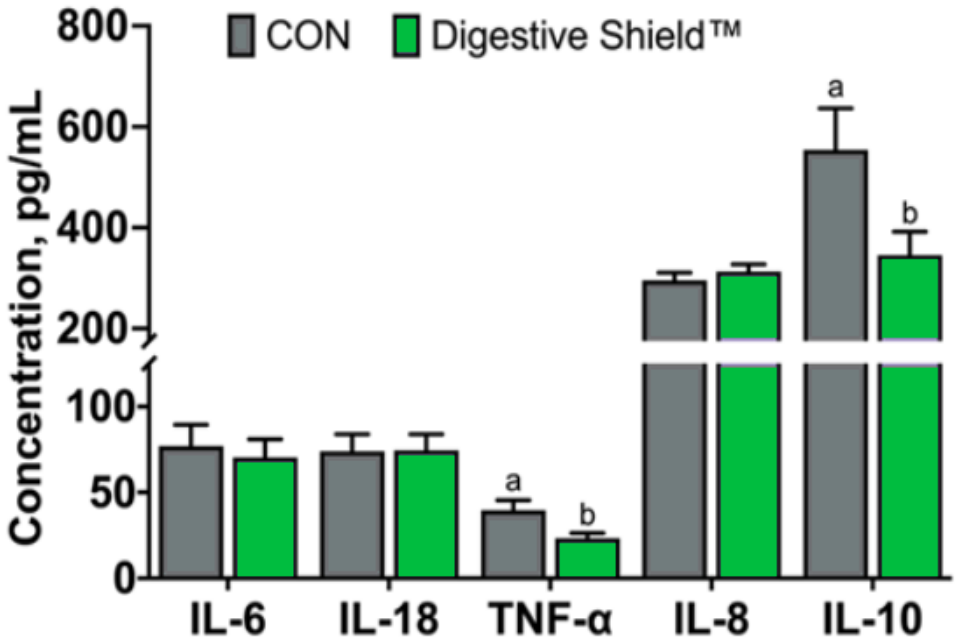


Figure 2. Whole-body inflammation markers in control (CON) and Digestive Shield™-fed horses following an antibiotic challenge [39].

The reduction in whole-body inflammation is further demonstrated by a reduction in malondialdehyde, a marker for oxidative stress, in healthy, exercised horses fed Digestive Shield™ compared to controls ($P < 0.05$) [40] since less inflammation can help lead to less oxidative stress. While some inflammation can be important in triggering the body's immune response, results from [38] indicate that healthy horses fed Digestive Shield™ may have their immune systems more primed and ready even with a reduction in inflammation. Both studies demonstrate that Digestive Shield™ may help healthy horses reduce whole body inflammation. Since inflammatory markers were reduced in both studies by at least 25% without negatively impacting key immune signaling markers, these results indicate an improved inflammatory response in healthy horses fed Digestive Shield™.

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