

# **Use of a Nutritional Supplement to Restore Equine Colonic Integrity.**

**An evaluation of a novel veterinary formula that promotes  
cell regrowth and mucus production in horses.**

A paper presented by:  
Freedom Health, LLC

Last revision:  
March 24, 2014

# Introduction

Gastric ulcers in horses are common, and are known to be associated with a variety of factors, including unnatural high-energy feeds, intermittent feeding and the stress of training. Less well known is that colonic ulcers are also common. The following research articles in this overview discuss the prevalence of colonic ulcers along with information on a patented natural formula that has been developed to address this poorly understood problem.

Over the course of a decade, one of the authors (Pellegrini) has performed several hundred necropsies of horses at abattoirs. The rates of colonic ulceration in these horses ranged between 60% and 80%. This is a primary indicator of ulcerative colitis, which may negatively impact both the health and performance of the horse.

In 2003 a natural, oat-based functional feed was formulated specifically to normalize the colonic environment. This formula, referred to as a Digestive Conditioning Program (“DCP”), was commercialized in 2004 and marketed directly to horse owners and trainers. DCP is a functional feed that is administered daily for at least a 90-day period. Controlled, blind tests of the formula in over 200 horses in training have shown significant improvement over controls, based on several markers of GI health. An analysis of these results is included in this overview.

In the intervening years, new understandings and approaches to colitis have been reported in the literature. Based on those studies, as well as our own, a new functional feed product has been formulated for veterinarian-only use, which we refer to as the Veterinary formula (“VF”). The VF formula is based on the original DCP, with added ingredients known to enhance cell regrowth and repair as well as mucus production. Using a multifaceted approach (CBC, serum amyloid, fibrinogen and fecal blood test), this formula has been shown to meet or exceed the efficacy of the original formula. The results of this study are also included in this overview.

# An introduction to colonic ulcers in horses

Franklin L. Pellegrini, DVM

By now, most horse veterinarians know that gastric ulcers are common in both foals and adult horses, with rates ranging from 60% in show horses to 90% in race horses.<sup>1</sup>

Because of its complicated and multifactorial nature, the term Equine Gastric Ulcer Syndrome (EGUS) was coined. Known correlatives include the stress of heavy training and unnatural feeding regimens – including grain-heavy diets and intermittent feeding – that are used to maximize performance and minimize cost.<sup>2</sup>

However, the damage done to the equine GI tract is not limited to the stomach. While the stomach is affected by factors that induce EGUS, it is just the first way-station; the problems continue throughout the entire GI tract. Carbohydrate loads in excess of what the small intestine can absorb continue to the hindgut, where a portion of them are fermented by bacteria into lactic acid, which can lead to hindgut acidosis.<sup>3</sup>

In over 1,000 necropsies of horses at abattoirs in Texas (U.S.A.) and Quebec (Canada), the author has found colonic ulcers at rates up to 84%.<sup>4</sup> The lesions are evidence of ulcerative colitis, an inflammatory disorder of the colon with poorly understood etiology and mechanisms. Along with pathologies such as laminitis and colic, this suite of symptoms represents a syndrome that, by analogy with EGUS, can be called ECUS for Equine Colonic Ulcer Syndrome. Although the exact causes are speculative, there is evidence to implicate carbohydrate loading and other abrupt dietary changes.<sup>5</sup> The incidence of colic is estimated at over 0.2 episodes per horse-year,<sup>6</sup> and colic is the number one killer of horses.<sup>7</sup>

Lesions and inflammation of the gut affect the absorption of nutrients and thus the overall well-being and performance of the horse. Ulcers and inflammation may be responsible for much of the subclinical anemia, listlessness, weight loss and general poor health noted by many performance horse veterinarians. In fact, the number one complaint about horses with ulcers is that their performance is declining.<sup>8</sup> With these animals, environment has a larger impact on their abilities than genetic factors.<sup>9</sup>

Natural remedies have been developed specifically to mitigate the negative effects that intense training and high-energy feeding has on the GI tract. One such product is called a Digestive Conditioning Program (DCP). When nutritional supplements are formulated to address specific bodily functions, it is called functional feeding. However, before any functional feed can be properly digested, the gut itself must be sound. Therefore, a feed that supports proper gut function is a necessary prerequisite for all other functional feeds. With the proper functional feeding program, the equine GI tract can quickly recover from insults even during strenuous training, allowing the horse to better absorb nutrients and operate at the peak of its abilities.

This overview includes a discussion of a large colonic ulcer study that motivated the development of a new functional feed called the Veterinary Formula (VF). This product, based on DCP, is designed to enhance normal healing of inflamed colonic tissue by

promoting cellular regrowth and mucus production. Also included in this overview is a study comparing this veterinary formula with the original DCP formula and a control group. Finally, there is a discussion of the ingredients in this new veterinary product.

## References

---

<sup>1</sup> McClure SR, Glickman LT, Glickman NW. *Prevalence of gastric ulcers in show horses*. J Am Vet Med Assoc 1999;215:1130-1133.

<sup>2</sup> Reese RE, Andrews FM. *Nutrition and Dietary Management of Equine Gastric Ulcer Syndrome*. Veterinary Clinics of North America: Equine Practice, Vol 25, Iss 1, April 2009, pp 79-92.

<sup>3</sup> Richards, N, Gn Hinch, and Jb Rowe. "The Effect of Current Grain Feeding Practices on Hindgut Starch Fermentation and Acidosis in the Australian Racing Thoroughbred." Australian Veterinary Journal 84, no. 11 (2006): 402–407. doi:10.1111/j.1751-0813.2006.00059.x.

<sup>4</sup> Pellegrini, Franklin L. "Results of a Large-Scale Necroscopic Study of Equine Colonic Ulcers." Journal of Equine Veterinary Science 25, no. 3 (March 2005): 113–117. doi:10.1016/j.jevs.2005.02.008.

<sup>5</sup> Hudson JM, Cohen ND, Gibbs PG, Thompson JA. *Feeding practices associated with colic in horses*. J Am Vet Med Assoc. November 15, 2001, Vol. 219, No. 10, Pages 1419-1425.

<sup>6</sup> Uhlinger C. *Investigations into the incidence of field colic*. Equine Vet J. Vol 24, iss S13, pp 16-18, Aug 1992

<sup>7</sup> Traub-Dargatz JL, Koprak CA, Seitzinger AH, Garber LP, Forde K, White NA. *Estimate of the national incidence of and operation-level risk factors for colic among horses in the United States, spring 1998 to spring 1999*. J Am Vet Med Assoc. 2001 Jul 1;219(1):67-71.

<sup>8</sup> Mitchell RD. *Prevalence of Gastric Ulcers in Hunter/Jumper and Dressage Horses Evaluated for Poor Performance*. Assoc. Equine Sports Med., September 2001.

<sup>9</sup> Kronfeld DS. *Speed Limit*. Dipl. ACVN, Dipl. ACVIM. March 2003 Article # 4212.

# A Nine-Year Necroscopic Summary of 524 Horses at Abattoirs

Franklin L. Pellegrini, DVM

## Abstract

This article discusses a series of gross necroscopic examinations totaling 524 horses from abattoirs in the United States and Canada over the eight year period from 2003 – 2011. The first examination revealed that, in addition to gastric ulcers at rates of 80%, horses also exhibited colonic ulcers at rates exceeding 60%. This is a symptom of ulcerative colitis, a problem long recognized in horses but, due to the impracticality of equine colonoscopies, difficult to diagnose until the condition becomes acute.

Ulcerative colitis may lead to poor performance in horses and is thought to contribute to colic, the number one killer of performance horses.<sup>1 2</sup> The summary described here aims to increase awareness of this important issue.

## Methods

In these studies, freshly euthanized horses from abattoirs in Texas, and later in Quebec, Canada, were necropsied. After the horses were euthanized, a fecal ball was collected to verify and refine various antibody fecal blood tests (FBTs) against gross observations. The horses presenting at the abattoir were mixed breeds and included riding, range, race and show horses, as well as animals bred for consumption. Immediately after euthanization, the digestive tract was removed and the stomach and colon were tied off for separate examination. The stomach was split open and a longitudinal incision was made along the entire length of the colon so they could both be laid out for observation.

Both gastric and colonic lesions were noted in the Quebec studies, they were broken down by colon quadrants and graded. Gastric ulcers were categorized according to the Practitioner's Simplified Scoring System<sup>3</sup> on a scale from 0 to 3:

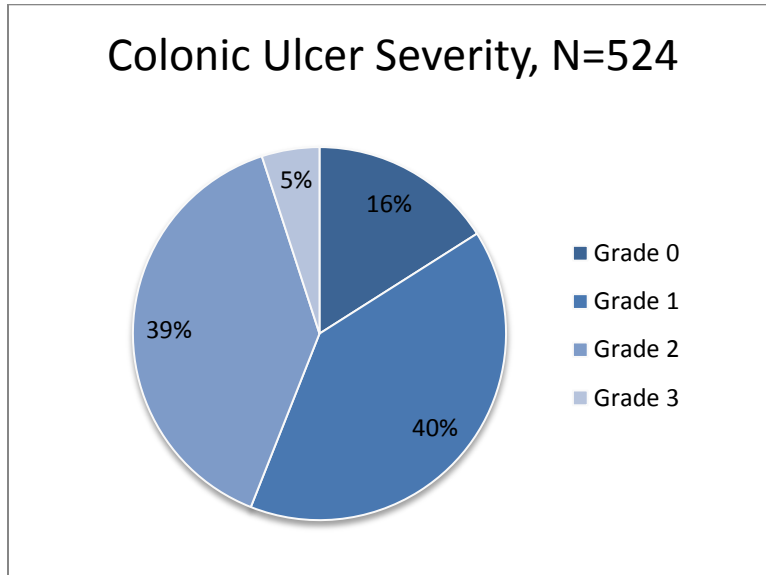
0. Intact mucosal epithelium (can have mild reddening and/or mild hyperkeratosis)
1. Small single or small multifocal lesions
2. Large single or large multifocal lesions or extensive superficial lesions
3. Extensive (often coalescing) lesions with areas of apparent deep ulceration

Due to the dearth of research on colonic ulcers, a corresponding colonic ulcer grading scale did not exist. We therefore applied the Practitioner's Simplified Scoring System to the colon for purposes of this study.

Although several different versions of the FBT were tested in these studies, the observations were collected using similar procedures. The data were collated from these necroscopic observations and grouped by equivalent protocols. The largest group yielded a population of 524 horses that could be categorized by ulcer grade and location.

## Results

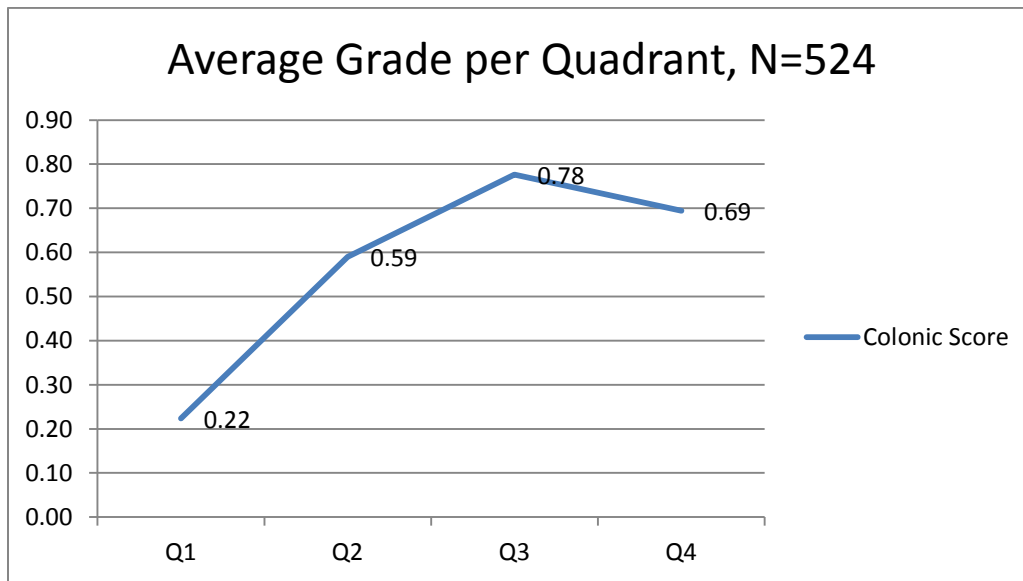
Only 16% of the animals were found to have a perfect score of zero with no noted lesions. Another 40% had small lesions, often petechiation and possibly caused by parasites. The number of horses with moderate to severe ulcers was 44%:



These scores were collected by colon quadrant according to this standard numbering scheme:

- Q1: left ventral colon
- Q2: left dorsal colon
- Q3: right ventral colon
- Q4: right dorsal colon

The colonic lesions were not equally distributed among these quadrants:



Notice that the score for Q4, the right dorsal quadrant, is not over-represented and in fact is less than the average score for Q3, the right ventral quadrant. Veterinarians familiar with right dorsal colitis (RDC) may be surprised to see the involvement in other quadrants. In this analysis, right-dorsal lesions account for less than a third of the visualized lesions. Although the treatment for RDC typically involves discontinuance of NSAIDs and feeding greater fiber, the treatment for these other ulcers of indeterminate etiology is unknown.

The ulcers noted over the years were diverse in type as well as location. Here is a small sample of the observations:



Ecchymoses, right dorsal colon



Cyathostomes (small strongyles), right dorsal colon



Intraluminal lipoma, left dorsal colon



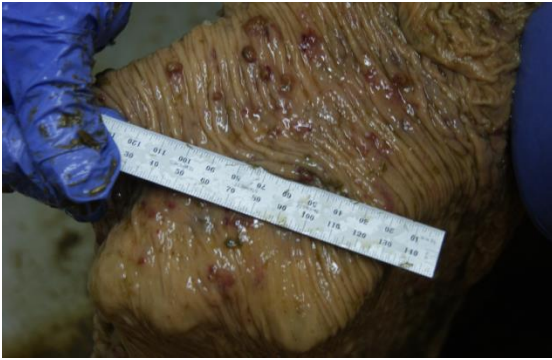
Well circumscribed focal pustule, left ventral colon



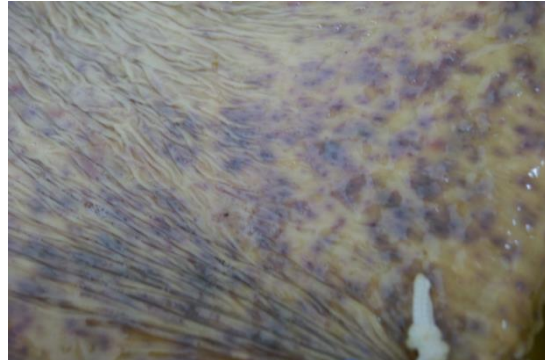
Pustule, left dorsal colon



Blood-filled bullae, right dorsal colon



Disseminated producing nodules, left ventral colon



Severe ecchymoses, left dorsal colon



Well-defined 2" x 3" bullae serosal surface, left ventral colon



Severe colonic ulceration, right dorsal colon

Many more colonic ulcers were observed than have been reported in the literature. The involvement of all four quadrants was intriguing, as was the diversity of lesions throughout the colon.

## Discussion

Equine Gastric Ulcer Syndrome (EGUS) has been extensively studied over the last few years, and the etiology for these gastric lesions is strongly correlated with modern high-energy feeding practices.<sup>4</sup> In order to excel at strenuous events like racing, the horse must be able to absorb unnatural quantities of energy-dense grains and carbohydrates. This leads to disruptions of the normal flow of digesta and the specific microfloral populations that have coevolved with the horse to deal with low-energy roughage.<sup>5</sup> In particular, increased carbohydrate loads can lead to hind-gut acidosis, laminitis and increases in certain acid-producing bacterial populations.<sup>6</sup>

Although gastric ulcers have received great attention, very little is known about the etiology of colonic ulcers other than NSAID-induced right dorsal colitis (RDC). Mostly this is due to the difficulty of performing colonoscopies on horses, whose lives are endangered by the lengthy preparative evacuation period. Horses that die of colic are not routinely necropsied, so complications and pathologies in this section of the GI tract are still poorly understood and difficult to diagnose until symptoms become acute.

Due to its large microbial population, the colon is vulnerable to infection and inflammation. The gut-associated lymphoid tissue (GALT) is the first line of defense and



– in keeping with the magnitude of its responsibility – it is the largest compartment of the immune system.<sup>7</sup> Ulcerative colitis is characterized by a degradation of the mucosa and underlying tissue, provoking an inflammatory response from the GALT. Inflammation anywhere in the body consumes energy and resources that are then unavailable to the horse, potentially affecting performance. Due to the high economic, health and performance impact of equine GI pathologies, it is important to better understand the prevalence and severity of ulcerative colitis in these animals.

Laboratory horses are expensive to keep and study, limiting the kind of research that can be done with them. The large necroscopic studies reported here were made possible by using euthanized horses from abattoirs in the United States and Canada. Over a period of nine years, the study has grown to encompass over one thousand horses. This review discusses the first 524 horses studied up to 2011. A more comprehensive meta-analysis incorporating the entire set of horses is currently underway.

This review demonstrates that colonic ulcers can be found in all four quadrants of the equine colon, with overall rates greater than 80%. It is likely that horses at an abattoir are stressed, and therefore may start to develop ulcers, which might explain some of the smaller lesions that were noted. However, lesions with scores greater than 1 were found in over 44% of the animals. Lesions of this severity are less likely to develop over the short period of time spent by horses at the abattoir and thus may represent a rough lower limit of ulceration for these cohorts.

These unexpectedly high rates and severities raise questions about the etiology of colonic ulcers, the effect of such a large rate of ulceration on health and performance, and the possible role of ulcerative colitis as a contributing factor in colic.

## References

---

<sup>1</sup> Bell, LG; Lowe, JE Incidence of major injuries, severe colic, and acute laminitis at American Horse Shows Association A- and B-rated shows. *J Am Vet Med Assoc.* June 1, 1986 v. 188 (11) p. 1304-1306.

<sup>2</sup> Traub-Dargatz JL, Koprak CA, Seitzinger AH, Garber LP, Forde K, White NA. Estimate of the national incidence of and operation-level risk factors for colic among horses in the United States, spring 1998 to spring 1999. *J Am Vet Med Assoc.* 2001 Jul 1;219(1):67-71.

<sup>3</sup> Andrews FM, Sifferman RL, Bernard W, Hughes FE, Holste JE, Daurio CP, Alva R, Cox JL (1999), "Efficacy of omeprazole paste in the treatment and prevention of gastric ulcers in horses", *Equine Vet. J.* 31: pp. 81–86.

<sup>4</sup> Reese RE, Andrews FM. *Nutrition and Dietary Management of Equine Gastric Ulcer Syndromw.* *Veterinary Clinics of North America: Equine Practice*, Vol 25, Iss 1, April 2009, pp 79-92.

<sup>5</sup> Richards, N, Gn Hinch, and Jb Rowe. "The Effect of Current Grain Feeding Practices on Hindgut Starch Fermentation and Acidosis in the Australian Racing Thoroughbred." *Australian Veterinary Journal* 84, no. 11 (2006): 402–407. doi:10.1111/j.1751-0813.2006.00059.x.

<sup>6</sup> Rowe, James B., Michael J. Lees, and David W. Pethick. "Prevention of Acidosis and Laminitis Associated with Grain Feeding in Horses." *The Journal of Nutrition* 124, no. 12 Suppl (December 1, 1994): 2742S–2744S.

<sup>7</sup> Brandtzaeg P. "Current topics in microbiology and immunology," (1989) Vol. 146, pp. 13-25. Ref: 48 *Journal code: 0110513. ISSN: 0070-217X. L-ISSN: 0070-217X.*

# A Nutritive Supplement for Veterinary Use

In order to mitigate the conditions leading to colitis in horses, Freedom Health LLC has created a new equine feed supplement to be sold exclusively through veterinarians, formulated to normalize disturbances in the GI tract while still allowing horses to be rigorously trained.

The veterinary formula described here contains ingredients that we believe improve upon the performance of the baseline retail product which has been commercially available since late 2004. The new formula includes Montmorillonite as a mycotoxin absorbent, mucin-related amino acids to enhance the formation of mucus, myoprotein-related amino acids to promote GI muscle tone, and B-vitamins to boost enterocyte metabolism.

The key ingredients of this formula include:

## Polar Lipids

Lipids represent a large class of molecules that include fatty acids, phospholipids (lecithin), galactolipids and triglycerides. They play a key role in the structure and function of cellular membranes and are found in much of the plant material already in equine diets. As a consequence of their ubiquity, lecithins and lipids are considered to be a GRAS (generally recognized as safe) supplement.

Oat oil is rich in polar lipids, particularly galactolipids. These are rare in animals, but are the most common lipids in plants as well as the most abundant form of lipids on the planet. Animals generally lack the enzymes needed to synthesize these polyunsaturated polar lipids, and so they must acquire them in their diet. Adding polar lipids to the diet has been shown to protect the intestinal mucosa and to strengthen the impermeability of the intestinal brush barrier.<sup>1 2 3</sup>

As well as galactolipids, oat oil is rich in trienols and tocopherols, powerful anti-oxidants that sweep free radicals out of the system before they can damage the surrounding tissue. These natural anti-oxidants also contribute to the shelf-life of the product.

Polar lipids are versatile emulsifiers, stabilizing oil-water mixtures. They provide an ideal nutrient delivery vehicle, capable of ferrying both fat and water-soluble molecules into the tissues. After transporting their nutritive load, polar lipids are readily absorbed in the gut (after digestion by bile salts), where they supply extra energy to the horse.<sup>4</sup>

Freedom Health uses polar lipids derived from specially extracted oat oil.

## Beta-glucan

Beta-glucan is a polysaccharide derived from yeast, barley and oats that has several profound effects on typical animal systems. It has been known for years to reduce LDL cholesterol levels in the blood.<sup>5 6</sup> More significantly, it moderates the release of sugars from the digestive system, helping to prevent the sugar highs and lows that often afflict animals that are fed only two or three times a day on an energy-rich high-carbohydrate

diet.<sup>7</sup> Studies have shown that beta-glucan is effective in reducing post-prandial glycemc peaks by up to 50%.<sup>8</sup>

Although more equine studies are needed, research has implicated bacteria in the formation of ulcers in many animals. While it has been difficult to culture bacteria from horse stomachs, colonic bacteria are generally known to include a wide variety of pathogenic species. Beta-glucan serves an important role here as a potent stimulator of the immune system. It arouses macrophages, which have a specific beta-glucan receptor, to mount a full-blown immune system response to pathogenic microbes and helping to heal damaged tissue.<sup>9 10 11</sup>

Beta-glucan also creates a gel, slowing the transit of digesta through the gut and allowing starches to be digested earlier in the system, thereby reducing the negative effects of starch in the hind gut.<sup>12 13</sup>

Freedom Health uses beta-glucan derived from oats.

## **Amino Acids**

Amino acids are the building blocks of proteins, and in the context of the intestines, they are important in maintaining the muscularis, the enterocytes and the mucosal layer.

Amino acids come in both a dextro- and levo- form, but only levo- forms are bioactive. Freedom Health only uses the levo- isomers of all its amino acids. We drop the “L-” designation in the following descriptions for the sake of brevity.

### ***Glutamine***

Glutamine is the most abundant amino acid in humans as well as horses. Glutamine deficits can result in diarrhea, villous atrophy, mucosal ulceration, increased intestinal permeability and necrosis.<sup>14</sup>

Glutamine is a muscle fuel and also supplies nitrogen to the immune cells of the intestinal mucosa, which help to prevent pathogenic organisms from entering the circulatory system. Glutamine can be administered parenterally to animals recovering from abdominal surgery to reduce healing time.<sup>15 16</sup>

Glutamine is considered to be a nonessential amino acid under normal conditions, because the body can create as much as is needed *de novo*. But Glutamine is also known to be “conditionally essential.” During periods of metabolic or digestive stress such as ulceration or malnutrition, large amounts of glutamine are consumed, and supplements may be needed to replenish the supply.<sup>17 18 19</sup> The intestines, along with immune and kidney cells, are the primary consumers of glutamine in animals.

### ***Threonine, Proline and Serine***

Threonine contributes to a smoothly functioning GI tract by assisting metabolism and nutrient absorption. A deficiency of threonine slows the regeneration of the gut wall and depresses the production of mucus.<sup>20 21</sup>

Threonine is especially useful for wound healing and for treating stress. It is also an essential link in the production of immunoglobulins.<sup>22</sup>

Threonine, proline and serine are the three most important amino acids in the mucoproteins that, along with sugars, make up mucus. Together, they account for over 40% of the total amino acids found in mucus.<sup>23 24</sup>

A continuous layer of mucus protects the lining of the gut and is produced by goblet cells distributed throughout the intestinal tract. However, bacteria and toxins can disrupt this mucus layer and damage the enterocytes lining the GI tract, leading to lesions and inflammation. In cases of ulcerative colitis, the mucus layer is denuded in the areas of greatest inflammation.<sup>25</sup> In order to maintain uninterrupted protection, extra mucus needs to be secreted. In times of stress, extra amino acids in the diet can be scavenged to help create new mucus quickly.

A specific mix of threonine, proline and serine has been shown to be beneficial in studies where dextran sulfate sodium was used to induce a model of ulcerative colitis. The extra amino acids increased the number of mucus-producing goblet cells at the ulcer sites. In addition, an optimal ratio of the three amino acids restored the gut microbiota to pre-treatment values.<sup>26</sup> We have reproduced the relative proportions of this restorative amino acid triplet in this formula.

### ***Leucine***

Leucine increases the post-feeding insulin response in horses. Both before and after exercise, leucine augments the rate of muscle glycogen synthesis.<sup>27</sup> Glycogen provides energy storage for muscles, including the muscles encasing the gut. Glycogen is always observed in the stratified epithelium of mucus membranes.<sup>28</sup> In addition, leucine is lost due to oxidation in the presence of *trichostrongylus colubriformis* (strongyles), leading to reduced growth rates.

### ***Isoleucine***

Serum isoleucine, along with leucine, is depleted after exercise and can take 48 hours to recover.<sup>29</sup> Isoleucine is a component of peptide histidine isoleucine (PHI), which is found throughout the digestive system, but particularly in the colon.<sup>30</sup> PHI, in turn, plays a role in the regulation of the immune system through its interplay with prolactin.<sup>31</sup>

### ***Lysine***

Lysine is an essential amino acid that has a major role in building muscle protein, in recovery from injury and is important to the growth of horses, especially yearlings.<sup>32 33</sup> Lysine and proline are important to GI healing and the formation of collagen.<sup>34</sup>

## **Montmorillonite**

Montmorillonite is a naturally occurring clay that absorbs a number of toxins, including aflatoxin, in the GI tract.<sup>35</sup> Animals (even humans) are known to seek out clay to help them detoxify their GI tract. When included in animal feed, Montmorillonite can ease GI disorders and has been used for years in both humans and animals.<sup>36</sup> *Aspergillus flavus* is the fungus that produces the mycotoxin aflatoxin, and it represents over 40% of the total mycobiota found in animal feed.<sup>37</sup> Horses are especially susceptible to mycotoxin poisoning, which can lead to abnormal behavior, depression, agitation and even dementia and blindness.<sup>38</sup>

Freedom Health uses food-grade Montmorillonite from natural sources.

## **Vitamins B<sub>6</sub>, B<sub>9</sub> and B<sub>12</sub>**

These B vitamins are involved in the cellular metabolism of amino acids, as well as proteins, carbohydrates and lipids. Because it is involved in heme synthesis, a deficiency of vitamin B<sub>6</sub> (pyridoxine) can lead to microcytic anemia.<sup>39</sup> Similarly, a deficiency of vitamin B<sub>9</sub> (folic acid)<sup>40</sup> or B<sub>12</sub> (cobalamin) can lead to macrocytic anemia.<sup>41</sup> Anemia may already be a problem in horses with bleeding GI lesions, so supplementation may be indicated.

## **Mannan Oligosaccharides**

Extracts of yeast (*saccharomyces cerevisiae*) have been found to bind pathogens,<sup>42</sup> perhaps because their mannan oligosaccharide (MOS) content mimics the carbohydrates in the enterocyte membranes. Pathogens may inadvertently bind with MOS instead of the enterocytes, and are subsequently flushed out of the digestive system. With the excretion of these pathogens, the toxins they would have produced are thereby avoided.<sup>43</sup>

MOS can be digested by the enzymes of certain beneficial bacteria. So, in addition to discouraging pathogenic bacteria, MOS also promotes the growth of beneficial microbes such as lactobacillus.

MOS also stimulates the immune system<sup>44</sup> and encourages the growth of intestinal villi, showing improved digestion and absorption of nutrients in various animal studies.<sup>45 46</sup>

Freedom Health uses MOS extracted from dried yeast.

## **Nucleotides**

Although the GI tract is covered in mucus, the acids and enzymes nevertheless take a toll on the enterocytes lining the gut wall. The cells there are constantly dividing, ultimately managing to totally replace the intestinal lining about every three days.

This continuous cell division requires the replication of millions of DNA molecules every second. In turn, each DNA molecule is made up of several billion nucleotides. This represents an impressive amount of energy-intensive chemical synthesis. Clearly, maintaining an adequate level of nucleotides is a major, ongoing problem for the digestive system.

In general, DNA is synthesized through a complicated pathway that creates fresh nucleotides de novo. However, in the presence of pre-made nucleotides, the body can down-regulate this synthesis and instead use an enzyme named HGPRT to scavenge the intact nucleotides, improving the efficiency of cell repair.

In addition to simple maintenance, cell division is also critical for the repair of damaged tissue, including ulcers. DNA synthesis is thus a limiting factor in the healing process as well.

In times of stress, certain cells of the digestive system – including the mucus-producing goblet cells – are incapable of meeting the increased demand for nucleotides. Under these conditions, nucleotide supplements in the diet have been proven to be beneficial. Studies

have shown that nucleotide supplements increase mucosal thickness and protein levels as well as speeding up intestinal recovery after chronic diarrhea and intestinal damage.<sup>47</sup>

Nucleotide supplements have been shown to increase the maturity and growth of normal enterocytes while reducing their dependence on exogenous glutamine.<sup>48</sup> The mechanism for this is not totally understood, but for intestinal villi to grow, stem cells in the crypts must divide and push their way up the length of the villi.<sup>49</sup> Exogenous DNA may enhance this process, perhaps explaining the extra crypt depth and increased surface area noted with nucleotide supplements.

Dietary nucleotides also seem to have an important beneficial effect on the intestinal microflora, stimulating the growth of beneficial bacteria and inhibiting pathogens. This may be due to yet another effect of dietary nucleotides reported in a NASA study and elsewhere: stimulation of the immune system.<sup>50 51</sup> In particular, lymphocytes and erythrocytes are not able to synthesize the purine-based nucleotides at all. For these cells, available nucleotides are essential to proper functioning.

Freedom Health uses nucleotides derived from dried yeast.

## References

---

- <sup>1</sup> Martin GP, Marriott C, Kellaway IW. *The interaction of progesterone with mucus glycoproteins*. Pharm Acta Helv. 1981;56(1):5-8.
- <sup>2</sup> Kiviluoto T, Paimela H, Mustonen H, Kivilaakso E. *Exogenous surface-active phospholipid protects Necturus gastric mucosa against luminal acid and barrier-breaking agents*. Gastroenterology. 1991 Jan;100(1):38-46.
- <sup>3</sup> McNeil PL, Ito S. *Gastrointestinal cell plasma membrane wounding and resealing in vivo*. Gastroenterology. 1989 May;96(5 Pt 1):1238-48.
- <sup>4</sup> Kreitler B. *Feed and Nutrition: Fat: The Next Nutraceutical?* Thoroughbred Times, April 12, 2003.
- <sup>5</sup> Davidson MH, Dugan LD, Burns JH, et al. *The hypocholesterolemic effects of beta-glucan in oatmeal and oat bran. A dose-controlled study*. JAMA 1991;265:1833-9.
- <sup>6</sup> Bell S, Goldman VM, Bistran BR, et al. *Effect of beta-glucan from oats and yeast on serum lipids*. Crit Rev Food Sci Nutr 1999;39:189-202 [review].
- <sup>7</sup> Braaten JT, Scott FW, Wood PJ, et al. *High beta-glucan oat bran and oat gum reduce postprandial blood glucose and insulin in subjects with and without type 2 diabetes*. Diabet Med 1994;11:312-8.
- <sup>8</sup> Tappy LE, Gugolz E, et al. *Effects of breakfast cereals containing various amounts of beta-glucan fibers on plasma glucose and insulin responses in NIDDM subjects*. Diabetes Care 19(8):831-4.
- <sup>9</sup> Czop JK. *The role of beta-glucan receptors on blood and tissue leukocytes in phagocytosis and metabolic activation*. Pathol Immunopahtol Res 1985;5:286-96.
- <sup>10</sup> Estrada A, Yun CH, Van Kessel A, et al. *Immunomodulatory activities of oat beta-glucan in vitro and in vivo*. Microbiol Immunol 1997;41:991-8
- <sup>11</sup> Reid DM, Montoya M, et al. *Expression of the beta-glucan receptor, Dectin-1, on murine leukocytes in situ correlates with its function in pathogen recognition and reveals potential roles in leukocyte interactions*. J Leukoc Biol 76(1):86-94.

- 
- <sup>12</sup> Bohm N, Kulicke W. *Rheological studies of barley (1-3)(1-4) beta-glucan in concentrated solution*, Carbohydrate Research, 1999, 315, 293-301.
- <sup>13</sup> Wursch P, Sunyer FX. *The role of viscous soluble fiber in the metabolic control of diabetes. A review with special emphasis on cereals rich in beta-glucan*. Diabetes Care 20(11):1774-80.
- <sup>14</sup> Roth E, Spittler A, Oehler R. *Glutamine: effects on the immune system, protein balance and intestinal functions*. Wien Klin Wochenshr. 1996;108(21):667-8.
- <sup>15</sup> Morlion BJ, Stehle P, Wachtler P, et al. *Total parenteral nutrition with glutamine dipeptide after major abdominal surgery: a randomized, double-blind, controlled study*, Ann. Surg. 1998, 227 (2): 302–8, DOI:10.1097/0000658-199802000-00022, PMC 1191250, PMID 9488531.
- <sup>16</sup> Rotting, AK, Freeman DE, Constable PD, Eurell JC, Wallig MA. Resistance of HOCl-injured mucosa recovered partially during the incubation period, and glutamine improved recovery. American Journal of Veterinary Research. November 2004, Vol. 65, No. 11, Pages 1589-1595. doi: 10.2460/ajvr.2004.65.1589.
- <sup>17</sup> Duckworth DH, Madison JB, et al. *Arteriovenous differences for glutamine in the equine gastrointestinal tract*. Am J Vet Res 53(10): 1864-7.
- <sup>18</sup> Vazquez P, Gomez de Segura IA, Cos A, Candela CG, De Miguel E. *Response of the intestinal mucosa to different enteral diets in situations of surgical stress and malnutrition*. Nutr Hosp. 1996 Nov-Dec;11(6):321-7.
- <sup>19</sup> Brosnan JT. *Interorgan amino acid transport and its regulation*, J. Nutr. 2003, **133** (6): 2068S–72S, PMID 12771367.
- <sup>20</sup> Bertolo RF, Chen CZ, Law G, Pencharz PB, Ball RO. *Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically*. J Nutr. 1998 Oct;128(10):1752-9.
- <sup>21</sup> Ball RO, Law G, Bertolo RFP, Pencharz PB. *Adequate oral threonine is critical for mucin production and mucosal growth by neonatal piglet gut*. Proceedings of the VIIIth International Symposium on Protein Metabolism and Nutrition, EAAP, 1999
- <sup>22</sup> Cuaron JA, Chapple RP, Easter RA. *Effect of lysine and threonine supplementation of sorghum in gestation diets on nitrogen balance and plasma constituents in first litter gilts*. J. Anim. Sci., 58, 631-637
- <sup>23</sup> Allen, A, and D Snary. “The Structure and Function of Gastric Mucus.” Gut 13, no. 8 (August 1972): 666–672.
- <sup>24</sup> Pearson, Jeffrey P., Raj Kaura, William Taylor, and Adrian Allen. “The Composition and Polymeric Structure of Mucus Glycoprotein from Human Gallbladder Bile.” Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology 706, no. 2 (September 7, 1982): 221–228. doi:10.1016/0167-4838(82)90490-3.
- <sup>25</sup> Pullan, R. D., G. A. Thomas, M. Rhodes, R. G. Newcombe, G. T. Williams, A. Allen, and J. Rhodes. “Thickness of Adherent Mucus Gel on Colonic Mucosa in Humans and Its Relevance to Colitis.” Gut 35, no. 3 (March 1, 1994): 353–359. doi:10.1136/gut.35.3.353.
- <sup>26</sup> Faure, Magali, Christine Mettraux, Denis Moennoz, Jean-Philippe Godin, Jacques Vuichoud, Florence Rochat, Denis Breuillé, Christiane Obled, and Irène Corthésy-Theulaz. “Specific Amino Acids Increase Mucin Synthesis and Microbiota in Dextran Sulfate Sodium-Treated Rats.” The Journal of Nutrition 136, no. 6 (June 1, 2006): 1558–1564.
- <sup>27</sup> Urschel, K. L., R. J. Geor, H L. Waterfall, A. K. Shoveller, and L. J. McCUTCHEON. “Effects of Leucine or Whey Protein Addition to an Oral Glucose Solution on Serum Insulin, Plasma Glucose and Plasma Amino Acid Responses in Horses at Rest and Following Exercise.” Equine Veterinary Journal 42 (2010): 347–354. doi:10.1111/j.2042-3306.2010.00179.x.

- 
- <sup>28</sup> Falin, L.I. "GLYCOGEN IN THE EPITHELIUM OF MUCOUS MEMBRANES AND SKIN AND ITS SIGNIFICANCE." *Cells Tissues Organs* 46, no. 3 (1961): 244–276. doi:10.1159/000141788.
- <sup>29</sup> Trottier, N. L., B. D. Nielsen, K. J. Lang, P. K. Ku, and H. C. Schott. "Equine Endurance Exercise Alters Serum Branched-chain Amino Acid and Alanine Concentrations." *Equine Veterinary Journal* 34, no. S34 (2002): 168–172. doi:10.1111/j.2042-3306.2002.tb05412.x.
- <sup>30</sup> Yiangou, Y, N D Christofides, M A Blank, N Yanaihara, K Tatemoto, A E Bishop, J M Polak, and S R Bloom. "Molecular Forms of Peptide Histidine Isoleucine-like Immunoreactivity in the Gastrointestinal Tract. Nonequimolar Levels of Peptide Histidine Isoleucine and Vasoactive Intestinal Peptide in the Stomach Explained by the Presence of a Big Peptide Histidine Isoleucine-like Molecule." *Gastroenterology* 89, no. 3 (September 1985): 516–524.
- <sup>31</sup> Kulick R, Chaiseha Y, Kang S, Rozenboim I, El Halawani M (2005). "The relative importance of vasoactive intestinal peptide and peptide histidine isoleucine as physiological regulators of prolactin in the domestic turkey". *Gen Comp Endocrinol* **142** (3): 267–273.
- <sup>32</sup> Ott, E A, R L Asquith, and J P Feaster. "Lysine Supplementation of Diets for Yearling Horses." *Journal of Animal Science* 53, no. 6 (December 1981): 1496–1503.
- <sup>33</sup> Graham, P. M., E. A. Ott, J. H. Brendemuhl, and S. H. TenBroeck. "The Effect of Supplemental Lysine and Threonine on Growth and Development of Yearling Horses." *Journal of Animal Science* 72, no. 2 (February 1, 1994): 380–386.
- <sup>34</sup> Thornton, Frank J., and Adrian Barbul. "HEALING IN THE GASTROINTESTINAL TRACT." *Surgical Clinics of North America* 77, no. 3 (June 1, 1997): 549–573. doi:10.1016/S0039-6109(05)70568-5.
- <sup>35</sup> "The Effect of Exchangeable Cations in Clinoptilolite and Montmorillonite on the Adsorption of Aflatoxin B1." Engormix.com. Accessed September 28, 2013. <http://en.engormix.com/MA-mycotoxins/articles/the-effect-exchangeable-cations-t95/p0.htm>.
- <sup>36</sup> Slamova, R., M. Trckova, H. Vondruskova, Z. Zraly, and I. Pavlik. "Clay Minerals in Animal Nutrition." *Applied Clay Science* 51, no. 4 (March 2011): 395–398. doi:10.1016/j.clay.2011.01.005.
- <sup>37</sup> Keller, K. M., B. D. Queiroz, L. a. M. Keller, J. M. M. Ribeiro, L. R. Cavaglieri, M. L. González Pereyra, A. M. Dalcerro, and C. a. R. Rosa. "The Mycobiota and Toxicity of Equine Feeds." *Veterinary Research Communications* 31, no. 8 (November 1, 2007): 1037–1045. doi:10.1007/s11259-007-0028-1.
- <sup>38</sup> Smith, T. K., and C. K. Girish. "The Effects of Feed Borne Mycotoxins on Equine Performance and Metabolism." (2008): 47–70.
- <sup>39</sup> Erslev, A. J., A. A. Lear, and W. B. Castle. "Pyridoxine-Responsive Anemia." *New England Journal of Medicine* 262, no. 24 (1960): 1209–1214. doi:10.1056/NEJM196006162622402.
- <sup>40</sup> Gross, R. L., J. V. Reid, P. M. Newberne, B. Burgess, R. Marston, and W. Hift. "Depressed Cell-mediated Immunity in Megaloblastic Anemia Due to Folic Acid Deficiency." *The American Journal of Clinical Nutrition* 28, no. 3 (March 1, 1975): 225–232.
- <sup>41</sup> R, Oh, and Brown DI. "Vitamin B12 Deficiency." *American Family Physician* 67, no. 5 (March 2003): 979–986.
- <sup>42</sup> Ip WK, Lau YL. *Role of mannose-binding lectin in the innate defense against Candida albicans: enhancement of complement activation, but lack of opsonic function, in phagocytosis by human dendritic cells.* *J Infect Dis* 2004 Aug 1;190(3):632-40. Epub 2004 Jun 28.
- <sup>43</sup> Swanson KS, Grieshop CM, Flickinger EA, Healy HP, Dawson KA, Merchen NR, Fahey GC Jr. *Effects of supplemental fructooligosaccharides plus mannanoligosaccharides on immune function and ileal and fecal microbial populations in adult dogs.* *Arch Tierernahr.* 2002 Aug;56(4):309-18.
- <sup>44</sup> Bland EJ, Keshavarz T, Bucke C. *The influence of small oligosaccharides on the immune system.* *Carbohydrate Research*, vol 339, issue 10.



- 
- <sup>45</sup> Newman, K. 1994. *Mannan-oligosaccharides: Natural polymers with significant impact on the gastrointestinal microflora and the immune system*. Biotechnology in the Feed Industry, Nottingham University Press, Nottingham, UK, pp. 167-174.
- <sup>46</sup> Davis E, Maxwell C, Kegley B, de Rodas B, Friesen K and Hellwig D, *Efficacy of Mannan Oligosaccharide (Bio-Mos) Addition at Two Levels of Supplemental Copper on Performance and Immunocompetence of Early Weaned Pigs*. Arkansas Animal Science Department Report 1999.
- <sup>47</sup> Bueno J, Torres A, Almendros A, Carmona R, Nunez MC and Gil A, (1994) *Effect of dietary nucleotides on small intestinal repair after diarrhea. Histological and ultrastructural changes*. Gut 35: 926-933.
- <sup>48</sup> Uauy R, Stringel G, Thomas R and Quan R, (1990) *Effect of dietary nucleosides on growth and maturation of the developing gut in the rat*. J. Pediatr. Gastroenterol. Nutr. 10:497-503.
- <sup>49</sup> Marshman E., Booth C., Potten CS., *The intestinal epithelial stem cell*. Bioessays 2002 Jan; 24(1):91-8.
- <sup>50</sup> Hales, N. *Diet Supplement May Help Boost Immune System*. American Society for Parenteral and Enteral Nutrition 25<sup>th</sup> Clinical Congress in Chicago.
- <sup>51</sup> Lin, Cheng-mao. *Effect of Dietary Nucleotide Supplementation on In Vivo and In Vitro Immune Function in Protein-Malnourished Mice*. University of Florida. PhD. Dissertation. December 1995.

# A 58-horse study of two formulas designed to normalize equine GI tract integrity

Franklin L. Pellegrini, DVM, and Vincenzo Franco, DVM, MRCVS

## Introduction

Since 2003, one of the authors, Dr. Pellegrini, has performed over 1,000 equine necropsies, concentrating on the GI tract, particularly the colon.<sup>1</sup> In addition to the well-known right dorsal colitis (RDC) brought on by overuse of NSAIDs, diverse lesions were also observed in all four quadrants of the colon. Based on these gross observations, the 20% of horses that had histories, and the existing literature, it seems likely that the dietary imbalances of a high-carbohydrate, high-performance diet bear some responsibility for suboptimal GI health, leaving it vulnerable to ulcers or inflammation.<sup>2 3</sup>

To address this issue, a nutritional supplement composed of natural ingredients, called a Digestive Conditioning Program (DCP), was designed to help maintain normal equine gut function, even in the face of unnatural (yet common) feeding and exercise regimens. Through a field trial with 80 horses in 2003, it was shown that horses fed this product showed improved fecal blood scores (using guaiac stain) and blood chemistries, proxies for improved intestinal health.<sup>4</sup> This product has been commercially available since late 2004.

Recently a new veterinary formula (VF) has been developed, based on DCP, but with the addition of B-vitamins to promote enterocyte metabolism and amino acids to support intestinal muscle tone and mucus production. This study was undertaken to evaluate the performance of the new veterinary formula as compared to the original DCP formula. On measures of whole-gut health, including blood chemistries and fecal blood tests, it is evident that VF equals or exceeds the supportive effect of DCP.

This study summarizes the results obtained over 120 days.

## Methods

59 performance horses in training were selected to conduct a controlled, randomized and blind study of two formulas, initiated in August, 2013. The two formulas in the study included DCP and VF, based on DCP but with added amino acids and vitamins. The horses were housed in three barns and placed on similar training and feeding regimens. Based on initial baseline CBC values and blood chemistries, the horses were sorted such that there were similar values for each cohort. All horses had water *ad libitum* and were fed TID on feed (Purina Edge) supplemented with grain. The horses were split into three randomly selected and blindly assigned cohorts. One horse in the control group fractured a 3<sup>rd</sup> carpal bone and was eliminated from the study, leaving the final sample size of 58 horses organized in three groups as follows:

1. Control: 18 horses (originally 19; one dropped out)
2. DCP: 20 horses
3. VF: 20 horses

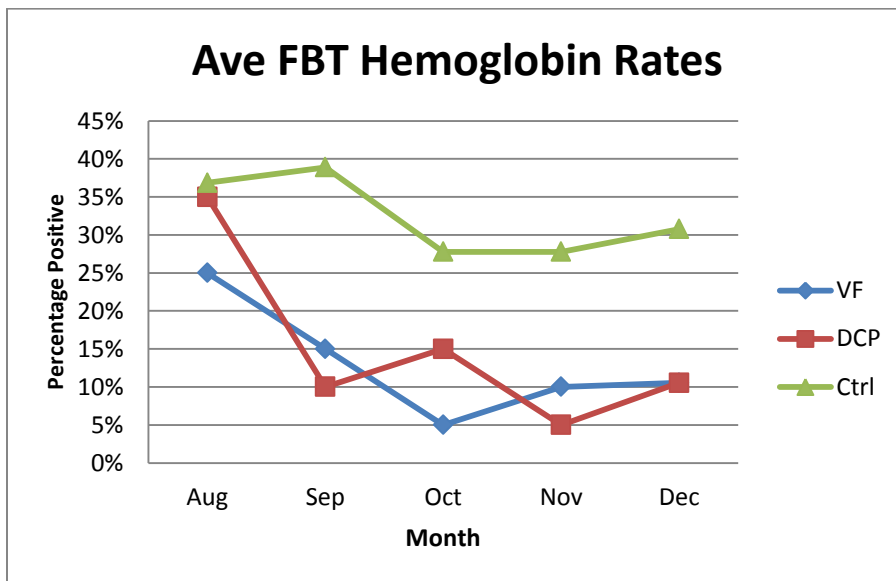
The two formulas were made to resemble each other physically and visually to avoid discriminatory bias. The study started with a fecal occult blood test to establish baseline GI health. The fecal occult blood test used is a rapid, sensitive antibody test that detects trace amounts of equine albumin and hemoglobin in the fecal matter which we have shown correlates to GI lesions.<sup>5</sup> Blood counts of red and white cells, standard blood panels (Na+, K+, tCO2, CK, Glu, Ca, TBIL, GCT, Alb, TP) and fecal pH were also analyzed each month and used in individual analyses of horse health. These analyses were scored as -1 if they tended toward a pathological condition, zero if homeostasis was observed and +1 if they returned toward normality from a pathological status.

## Results

Test results were recorded monthly for each horse during the study, including standard blood chemistries and counts as well as fecal tests for blood proteins and fecal pH, in order to independently analyze the health of each horse.

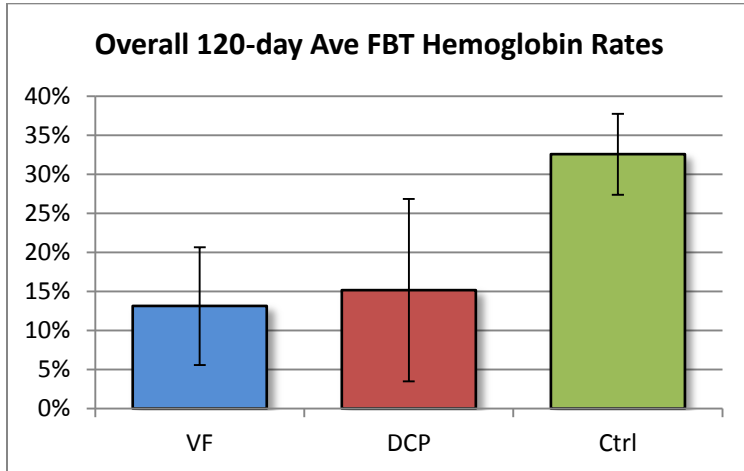
### Hemoglobin

Fecal hemoglobin positives in both formula groups showed improvement (lower average incidence rates) over the four-month period of the study. Lowered rates of hemoglobin in fecal matter may indicate a diminution of GI permeability, a common source of normal blood loss. In more severe cases, hemoglobin in feces may be an indicator of ulcers and/or inflammation in the stomach or intestines.



Average hemoglobin fecal blood test percentages over four months.

For both formulas, incidence rates of positive results for fecal hemoglobin were lower than the control group for all four months of the study. Averaged over the entire four-month period, the hemoglobin percentages are easy to compare:

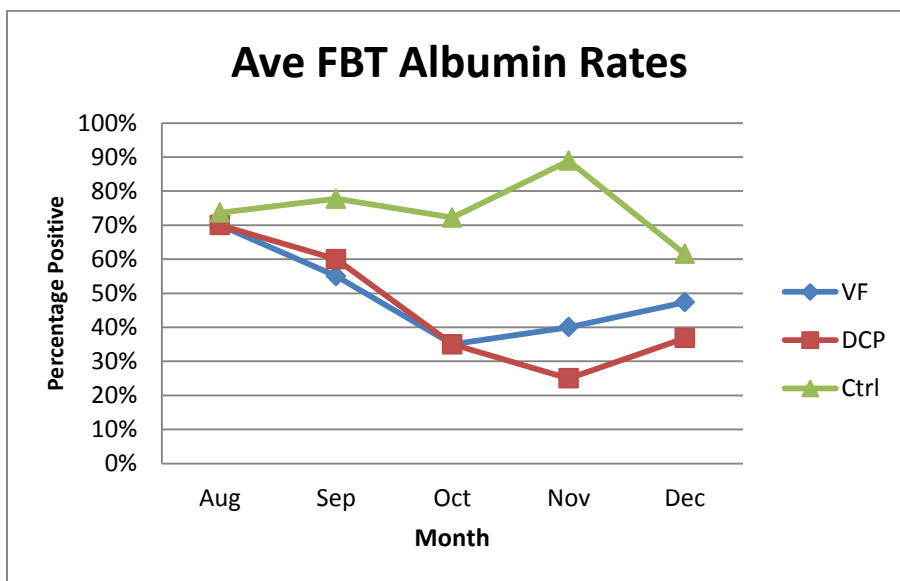


Overall 120-day averaged percentages of fecal blood test hemoglobin.

For both VF and DCP, fecal hemoglobin percentages are lower than the control. The vertical lines indicate standard deviation for each data set. Here it can be seen that VF shows less standard deviation than DCP in the collected FBT scores and that VF ranks significantly lower than the controls, while DCP and the control are closer when the deviation is taken into account.

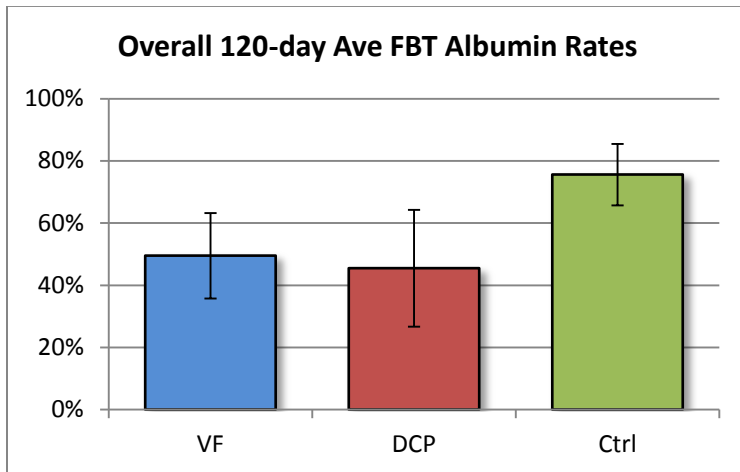
**Albumin**

Albumin is degraded by pepsin and other enzymes in the stomach<sup>6</sup> and in the proximal small intestine, so lowered rates of fecal albumin positives indicate a diminution of intestinal permeability or bleeding caudal to the common bile duct. In more severe cases, this can be an indicator of colitis or enteric lesions. Our past studies<sup>7</sup> have shown that this test correlates well with colonic lesions. Over the four months of the study, the percentage of albumin positives detected in the horses in either test group (DCP or VF) dropped significantly from the control level.



Average albumin fecal blood test percentages over four months.

Positive tests for albumin in the feces showed a consistent decline over the four-month period of the study. Again, we can view this as an average over the whole four-month study.

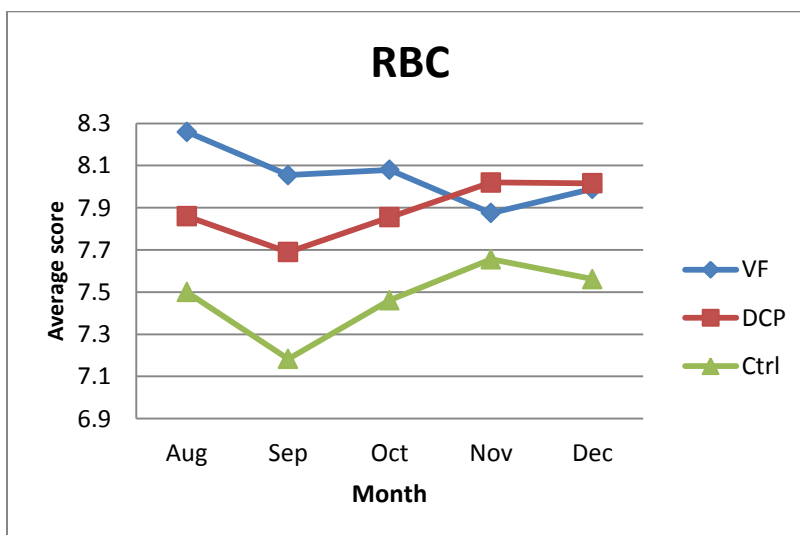


Overall 120-day averaged percentages of fecal blood test albumin.

Here, both DCP and VF groups demonstrate similar drops in FBT albumin detection levels, and again the standard deviation for VF is smaller than for DCP.

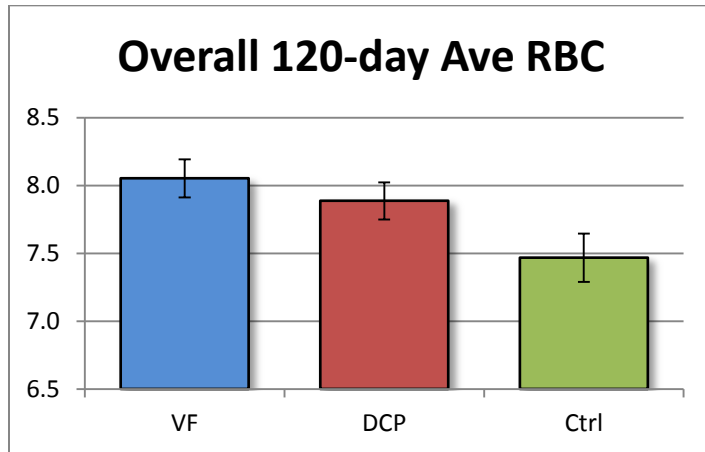
**Red blood cell counts**

A decrease in serum red blood cell counts may correlate with increased levels of intestinal permeability, inflammation or ulceration, a common cause of blood loss.<sup>8</sup> The following graphs show serum RBC counts over each month of the study.



Red blood cell counts over four months.

The RBC counts were consistently higher for both VF and DCP than the controls over the entire period. Again, we can see the overall effect by looking at 120-day averages.

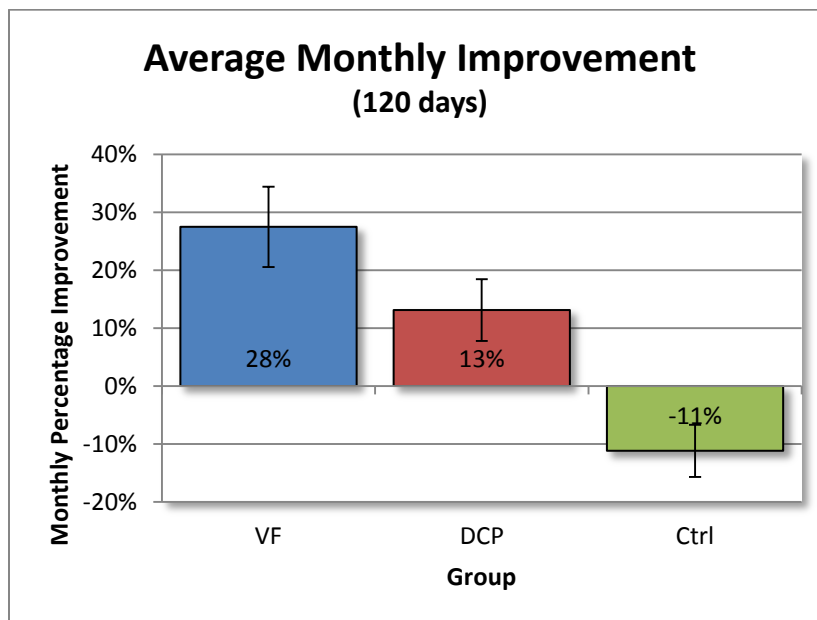


Overall 120-day average red blood cell counts.

The best results were from VF, although both VF and DCP showed a significant separation from the control, as seen by their standard deviations.

### **Individual assessments**

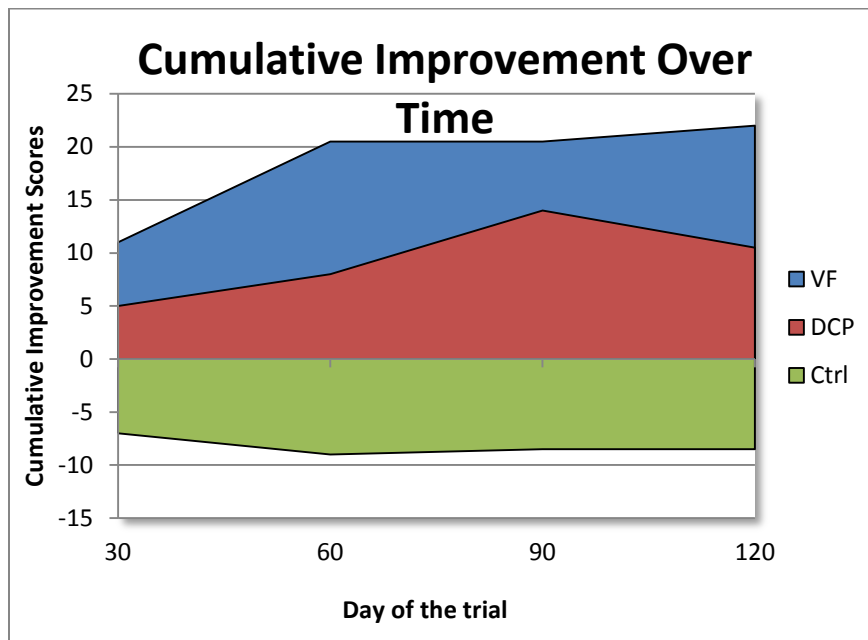
Finally, the health of individual horses was examined to determine which ones in each group had changed in each monthly period. With the groups blinded, and based on the FBT results, blood chemistries and CBCs, both authors of this paper prepared independent assessments of every horse, scoring them -1, 0 or 1 to indicate decline, no change, or improvement in health as compared to the previous month. This was repeated for each month of the study after the first month, which was used to establish the initial baseline. The independent assessment scores for each author (which were in close alignment, differing only by a single horse) were averaged and summed over each group.



The monthly improvement scores averaged over the entire 120-day study.

Improvements were observed in an average of 28% of the horses in the VF formula cohort in each monthly assessment, which exceeded the improvement noted in the DCP formula cohort. The difference in the VF and DCP cohorts exceeded the standard deviation of each measure, emphasizing the significance of the difference. The generalized decline in the health assessments of the control cohort is something that has been observed in these studies before.<sup>9</sup> It may represent an increased GI permeability or inflammation during the intensive training, transport and performance routines of these horses during the study period.

This can also be viewed as a cumulative chart over time, where the number of horses that improved in each period is added to the next.



The cumulative improvement in individual horses over the 120-day study.

It can be seen from this that the overall health of horses in the cohort fed SUCCEED VF improved quickly by 60 days, and continued to improve or remain equal over the next 60 days.

## Discussion

In this study, a new functional feed targeting GI integrity and health has been shown to reduce measures associated with gastric and colonic lesions and other aspects of GI health and overall health. This veterinary formula (VF) is based on DCP, but with ingredients added to improve muscle tone, enterocyte replenishment and mucus production.

Based on CBCs, blood chemistry and results of an antibody test for fecal hemoglobin and albumin, both formulations, VF and DCP, were shown to improve scores related to intestinal health as compared to the control group. On individual assessments of horse health, VF scored better than the original formula in three out of four months of the study.

Based on these results, veterinarians should expect a similar improvement of gut health, greater circulating RBCs and improved protein levels. Each of these improvements contributes to the overall health and athleticism of the horse.

Based on rates of fecal blood components – albumin and hemoglobin – found in the horses and in previous necroscopic studies,<sup>10</sup> it is likely that some of these horses had gastritis or colitis. It is still unclear exactly how colitis relates to colic in horses, but researchers generally recognize three main potential precursors to colic<sup>11</sup>:

1. colitis or diarrhea
2. obstruction or torsion of the intestine
3. intestinal impaction or enteroliths

To the extent that colitis leads to colic, and to the extent that colitis can be mitigated with a functional feed like VF, veterinarians may find it possible to reduce the rate of colic in their equine patients.

## References

---

- <sup>1</sup> Pellegrini, FL. “Results of a Large-Scale Necroscopic Study of Equine Colonic Ulcers.” *Journal of Equine Veterinary Science* 25, no. 3 (March 2005): 113–117. doi:10.1016/j.jevs.2005.02.008.
- <sup>2</sup> Reese RE, Andrews FM. *Nutrition and Dietary Management of Equine Gastric Ulcer Syndrome*. *Veterinary Clinics of North America: Equine Practice*, Vol 25, Iss 1, April 2009, pp 79-92.
- <sup>3</sup> Richards, N, Gn Hinch, and Jb Rowe. The Effect of Current Grain Feeding Practices on Hindgut Starch Fermentation and Acidosis in the Australian Racing Thoroughbred. *Australian Veterinary Journal* 84, no. 11 (2006): 402–407. doi:10.1111/j.1751-0813.2006.00059.x.
- <sup>4</sup> Pellegrini FL, Bedding P. *Veterinarian Information*, Freedom Health LLC, Jan 2006.
- <sup>5</sup> Ibid.
- <sup>6</sup> Weber G, Young LB, *Fragmentation of Bovine Serum Albumin by Pepsin*. *J Bio Chem*, May 1964, Vol 239, 1415-1423.
- <sup>7</sup> Pellegrini, *Veterinarian Information*, Op cit.
- <sup>8</sup> Andrews FM, *Overview of Gastric and Colonic Ulcers*, *Advances in Equine Nutrition IV*, 2008
- <sup>9</sup> Pellegrini, *Veterinarian Information*, Op cit.
- <sup>10</sup> Pellegrini, *Veterinarian Information*, Op cit.
- <sup>11</sup> Ib, Johnstone, and Crane S. “Hemostatic Abnormalities in Equine Colic.” *American Journal of Veterinary Research* 47, no. 2 (February 1986): 356–358.