

NUTRITION AND THE EQUINE FOOT

PETER HUNTINGTON¹ AND CHRIS POLLITT²

¹*Kentucky Equine Research Australasia*

²*Australian Equine Laminitis Research Unit, Queensland, Australia*

Many performance and racehorses cannot perform to their potential because of hoof problems. The old adage “no hoof, no horse” still applies today and this article examines some of the nutritional factors that impact the hoof. There are many factors that can influence development of the hoof, and this article will discuss some of these variables that can aid the search for increased hoof growth and improved hoof quality. Unfortunately, rapid hoof wall growth may not be synonymous with top hoof quality.

Despite recent advances in the prevention or treatment of equine disease, laminitis remains high on the list of potentially crippling or life-threatening diseases. The second part of the article will summarize new research findings in the cause, pathogenesis, prevention, and treatment of laminitis.

Factors Affecting Hoof Growth

Hoof growth is influenced by several factors. These include age, breed, genetics, metabolic rate, exercise, external temperature, environmental moisture, illness, trimming, and shoeing. Nutritional influences include energy intake, protein and amino acid intake and metabolism, minerals such as zinc and calcium, and vitamins such as biotin and vitamin A.

Moisture has an influence on both hoof growth and strength of hoof horn. Growth is often increased in wet conditions, and this could be a primary effect from increased hoof moisture or a secondary effect of greater pasture growth and energy intake. However, it has been demonstrated that poor-quality, softer horn has a higher water content (Coenen and Spitzlei, 1997). Butler and Hintz (1977) showed that hoof moisture in growing ponies dropped from 29% at the coronary border to 27% at the tip of the toe, and this was associated with, but not necessarily responsible for, a 30% increase in hoof strength between the two areas.

Energy Intake

When faced with bad feet, the first thing to consider when evaluating a feed program is total feed (energy) intake. Meeting energy requirements may be the

first and most important step in ensuring hoof growth and integrity. A horse in negative energy balance will utilize protein in the diet or body to make up energy needs for maintenance or growth. This may create a secondary protein or amino acid deficiency.

Butler and Hintz (1977) showed that hoof wall growth was 50% greater in growing ponies in positive energy balance than in ponies on restricted diets with reduced body growth rate. But the restriction in energy, protein, and mineral intake did not reduce hoof wall strength. It is a common observation that when horses gain weight on lush spring grass they also grow hoof faster.

Hoof tissue contains about 3-6% fat to bind some of the cells together and to help repel water. Recent research has shown that increasing the dietary intake of fat has little effect on hoof growth rate or strength (Lewis, 1995; Ott and Johnson, 2002), but fat can be a valuable addition to the diet for other reasons such as maintaining positive energy balance or coat conditioning.

Protein and Amino Acids

The hoof wall is about 93% protein on a dry matter basis. Because of the composition of the hoof wall, most of the commercially available hoof supplements contain methionine. However, methionine is just one of the amino acids contained in the protein of the hoof, and deficiencies of any essential amino acid can be as detrimental as a deficiency of methionine. Hoof contains high levels of cystine, arginine, leucine, lysine, proline, serine, glycine, and valine, and lower levels of methionine, phenylalanine, and histidine (Samata and Matsuda 1988; Coenen and Spitzlei, 1997). Coenen and Spitzlei compared the amino acid content of normal hoof and horn of poor quality. They found a linear correlation between cystine content and hardness in normal horn but not in poor-quality horn. The protein of normal horn contained higher levels of threonine, phenylalanine, and proline and lower levels of arginine than poor-quality horn.

Ekfalck et al. (1990) showed there was a clear difference between the distribution of the two sulfur-bearing amino acids in the keratinizing epidermis of the hoof. Cystine was located mainly in keratinocytes of the keratogenous zone in the matrix and in the nucleated keratinocytes that formed the incompletely keratinized basal part of the primary epidermal laminae and covered the lateral surface of the outer, fully keratinized part of those laminae. Methionine was located primarily in the stratum basale and in the stratum spinosum of the matrix and in the secondary epidermal laminae of the laminar layer. The pathway that converts methionine to cysteine is thought to be imperative in the production of quality hoof.

Protein-deficient diets lead to reduced hoof growth and splitting and cracking of the hoof (Lewis, 1995). However, Richardson and Ott (1977) looked at the influence of amino acid intake and found that diets intended to support more

rapid growth of young horses did not necessarily maximize hoof growth. They showed that brewer's dried grains, a protein source with a lysine content low enough to restrict growth in young horses, led to significantly increased hoof growth compared to diets based on soybean meal. In another study at the University of Florida, 39 Thoroughbred and Quarter Horse yearlings were used in two 112-day experiments to determine the effect of lysine and threonine supplementation on growth and development (Graham et al., 1994). The addition of 0.2% lysine to a basal diet led to an increase in growth, and this effect was enhanced by an extra 0.1% threonine; however, no changes were seen in hoof growth due to diet. This suggests that the amino acid needs for body growth and hoof growth are different.

Gelatin is a protein source used to treat fingernail growth abnormalities in man. Research has revealed that gelatin supplementation has no influence on hoof growth or quality. Butler and Hintz (1977) also examined the effects of gelatin in the diet of growing ponies and found no impact on hoof growth or quality. Goodspeed et al. (1970) reported that the addition of 114 g of gelatin per day added to a growing ration reduced hoof specific gravity but had little effect on hoof tensile strength.

Minerals

Current thinking on the relationship of diet and hoof integrity puts too much emphasis on zinc and too little emphasis on the other minerals necessary for metabolism. The health of the foot is an extension of the health of the horse, and if mineral deficiencies compromise horse health in general, then the health of the foot is going to be negatively impacted as well. There is justification for looking specifically at zinc when trying to put together the "hoof healthy" diet. Zinc is involved in the health and integrity of hair, skin, and hoof, but adding additional zinc to a diet that is already adequate in zinc is not going to automatically result in any dramatic increases in hoof quality or growth rate.

Coenen and Spitzlei (1997) have shown that 25 horses with poor horn quality have lower blood and hoof zinc levels than 38 horses with normal feet. There was no difference in the levels of copper and selenium in the same horses. This may be due to individual zinc absorption, metabolism, or retention abnormalities. The same study showed that supplementation with 300-500 mg zinc per day led to an increase in the zinc content of the horn. Butler and Hintz (1977) found that limited ponies with reduced hoof growth had significantly higher zinc levels in hoof horn, but there was no correlation with strength or elasticity. A recent study in Japan reported that horses consuming diets low in zinc and copper were more likely to have white line disease than horses that were supplemented with higher levels of these trace minerals (Hihami, 1999). The form of zinc in the diet may have some relevance to the poor-footed horse as chelated zinc may produce results when inorganic zinc does not work. Chelated zinc contains zinc bound to an

amino acid, and the zinc is absorbed with the protein, which potentially enhances absorption. Chelated zinc is used widely in dairy cattle to improve hoof strength, and most hoof supplements contain chelated zinc.

Ott and Johnson (2001) examined the effect of mineral source on growth and hoof development of yearling horses. Fifteen yearlings were fed one of two diets for 112 days. Diet A provided NRC or higher levels of all of the nutrients with trace minerals provided in an inorganic form. Diet B provided the same amount of each mineral except that supplementary zinc, manganese, and copper were added as proteinate. Trace mineral source had no effect on feed intake, nutrient intake, or feed efficiency. Growth of the yearlings was generally not influenced by source of mineral except for hip height, which was greater for the proteinate-supplemented yearlings. Hoof growth was significantly greater for colts when compared to fillies and for proteinated minerals compared to inorganic minerals. The increase in hoof growth due to the proteinated minerals was about 4%. Breaking strength of the hoof was greater for Quarter Horses than Thoroughbreds but was not influenced by sex or feeding.

In another study, Siciliano et al. (2001) compared supplementation with inorganic and organic sources of manganese, zinc, and copper in adult mares where 50% of the trace mineral needs were supplied by chelated minerals. No differences in hoof growth rate, hoof hardness, and tensile strength were detected. Hoof wall trace mineral content was not influenced by the diets. The differences between these two variable results may be due to the age of the animals or the amount of chelated minerals used. Growing animals are likely to exhibit a greater demand for nutrients needed for growth and may not have the option of providing extra minerals for hoof development.

Although calcium is only present at 300-350 mg/kg hoof wall, it is involved in creating the sulfur cross-links between the hoof proteins and in the cohesion of cells to each other. Kempson (1987) reported that 31 of 33 horses with brittle feet had a loss of the tubular structure in the stratum medium and internum. Twenty of these horses had failed to respond to biotin supplementation, but the majority showed an improvement when the protein and calcium intakes were increased. These horses were reported to be on diets of oats or bran and chaff or grass hay. Supplementation with lucerne supplied extra calcium and boosted protein and amino acid intake, so the exact cause of the improvement cannot be determined. If calcium levels in the diet are low, then supplying extra calcium may positively impact hoof quality as well as bone quality.

Selenium is the mineral with the narrowest safety margin between the requirement and toxic levels. Signs of toxicity include loss of hair, lameness, hoof rings and cracks, and separation of hoof walls (Lewis, 1995). This can be due to pastures that accumulate selenium such as those found in areas of the western United States or central Queensland. There are also reports of selenium toxicity due to inadvertent overdoses from premixes in feed in England or oral and injectable supplements in New Zealand.

Biotin

Most of the emphasis on research on hoof growth and hoof wall quality has involved the vitamin biotin. It is thought that the normal horse has a biotin requirement of 1-2 mg per day, and this can be supplied in certain feedstuffs as a component of commercial vitamin and mineral premixes or by intestinal synthesis by microorganisms in the large intestine. Biotin is a cofactor in a number of enzyme systems. In other animals, chronic biotin deficiencies lead to lesions of the skin and other keratinized structures, and supplementary biotin was first used in pigs to treat hoof problems. Studies have shown that supplemental biotin at levels of 15-20 mg per day has had positive effects on hoof quality in some horses and may increase hoof growth but does not assist all horses. Biotin is the most expensive vitamin to supplement and patience is necessary as it takes 9-12 months to grow an entire new hoof.

Comben et al. (1984) published one of the first case studies after having extrapolated the dose of biotin from the breeding sow. They used 15 mg for Thoroughbreds and 15-30 mg for draft horses with poorly shaped hooves, cracks in the hoof wall, and soft and crumbling horn. After five months of treatment, the hoof horn was thicker and harder, and improvement continued when the horses were examined another four months later. Shoeing was easier and shoes lasted longer as there was more strong horn onto which the shoe could be nailed.

Josseck et al. (1995) reported a controlled, double-blind study in which 42 600-700-kg Lipizzaner stallions with poor-quality hooves were treated with 20 mg biotin per day for over three years. Assessments of hoof quality were made and compared to control horses. This study showed that it took six months for appreciable differences between treated and control horses and nine months to achieve a statistically significant difference. Some horses did not improve until more than one year after treatment began, but hoof horn quality continued to improve as the period extended beyond 18 months. Biotin treatment reduced the incidence and severity of hoof horn defects, increased tensile strength, and improved condition of the white line. Significant changes in tensile strength were not seen until 33 months after treatment began. Treated horses had plasma biotin levels of over 1000 ng/ml compared to a mean level of 350 ng/ml in untreated horses. In this experiment, however, biotin supplementation did not increase hoof growth rate. Zenker et al. (1995) reported histological findings from the Lipizzaner study and found a significant reduction in the horn abnormalities in treated horses only after 19 months of treatment. The major pathological changes were microcracks in the transition from the middle to the inner zone of coronary horn and separation of the sole from the coronary horn in the white line.

Buffa et al. (1992) gave differing doses of biotin to horses and compared the effect on hoof growth rate and hardness in groups of eight horses. Horses were treated for 10 months with a placebo, 7.5 mg per day, 15 mg per day, or 15 mg

daily in alternate months. All treatment groups had significantly greater hoof growth and hardness than control horses, with best results in the horses getting 15 mg per day. The increase in hardness was more apparent at the toe and quarters than at the heel. Seasonal influences were seen in hoof hardness, and all hooves were harder in the dry season.

Geyer and Schulze (1994) conducted a long-term study on the influence of dietary biotin in horses with brittle hoof horn and chipped hooves. The study was performed over periods from one to six years. Ninety-seven horses received 5 mg of biotin per 100 to 150 kg of body weight daily; 11 horses were not supplemented with biotin and served as controls. The hooves of all horses were evaluated macroscopically every three to four months and horn specimens of the proximal wall were examined histologically and physically in 25 horses. The hoof horn condition of the biotin-supplemented horses improved after eight to 15 months of supplementation, but the hoof horn condition of most control horses remained constant throughout the study. The hoof horn condition deteriorated in seven of 10 horses after biotin supplementation was reduced or terminated. The horn growth rate of treated horses and of control horses was the same.

Reilly et al. (1998) used a higher dose rate of biotin in a controlled feeding trial. They examined the effect of 0.12 mg/kg body weight on growth and growth rate of the hooves of eight paired ponies. After five months, treated ponies had a significantly faster mean hoof growth at the midline of 35.34 mm, compared to control animals' 30.69 mm. Comparison of regression analysis also showed that biotin supplementation produced a significantly higher growth rate of hoof horn in this trial. Treatment animals had a 15% higher growth rate of hoof horn and 15% more hoof growth at the midline. The positive effect on hoof growth seen in this study may be due to the higher dose of biotin used, which equates to 60 mg per day in a 500-kg Thoroughbred horse.

There is evidence that adequate amounts of vitamin A in the diet may be important in promoting normal hoof wall growth. Vitamin A is involved in maintaining epithelial integrity and may have an important role in cell maturation and differentiation in the foot. Vitamin A is present in green grass, new hay, commercial feeds, and supplements. Deficiencies are unlikely given that vitamin A is present in many feeds and is stored in the body for some time but can occur in dry conditions or in horses on an unsupplemented diet.

The Bottom Line on Improving Hoof

Even though most poor feet are a result of genetic factors and bad mechanics, there is a piece of the riddle that can be solved with good nutrition. Use a feed that is designed for the class of horse you are feeding, and feed enough of it to get the desired body condition. Look for feeds that are balanced for macro- and microminerals. Zinc and calcium are critical for hoof growth and strength. Apart from special feed concentrates which are designed to be fed with oats, commercial

feeds should not be cut with oats as this wrecks the nutrient balance the nutritionist has attempted to achieve. In addition to a good feed, emphasis should be placed on high-quality hay such as lucerne (alfalfa). Sometimes it is necessary to restrict energy intake in specific diets. In these cases, it is important to make sure requirements for the other nutrients are met.

If everything is being done from nutritional and farriery (shoeing/trimming/hoof dressing) angles and hoof quality is still poor, it is worth experimenting with supplemental biotin, methionine, and zinc. Unfortunately, there is no quick fix and maintaining a good foot on a horse is a combined result of good farriery, good nutrition, good health care, and selecting for horses that genetically have healthy hooves.

Laminitis

Laminitis is the most serious disease of the equine foot and causes pathological changes in anatomy that lead to long-lasting, crippling changes in function. It is the second biggest killer of horses after colic. In the National Animal Health Monitoring System (NAHMS) report for the year 2000 in the United States, 13% of all horse operations (excluding racetracks) had a horse with laminitis in the previous year and 4.7% of these animals died or were euthanatized. More horses were affected by laminitis in spring and summer than in winter. Grazing lush pasture and grain overload was the cause of 50% of the laminitis cases reported. The report concluded that proper grazing and feed management could prevent approximately 50% of laminitis cases. Grass founder is thus a major cause of laminitis in the United States, and the same is probably true of most temperate regions of the world. There is anecdotal evidence that selective breeding for high fructan concentration in pastures designed for ruminants is increasing the incidence of grass founder in horses in the United States (Watts, 2001) At the Australian Equine Laminitis Research Unit, we have been studying how fructan from plants induces laminitis, discovering how and when pasture species produce dangerous levels of fructan, and pinpointing which pasture species produce the most fructan.

Laminitis has a developmental phase, during which lamellar separation is triggered. This precedes the appearance of the foot pain of laminitis (Moore et al., 1989). The developmental period lasts 40-48 hours in the case of laminitis caused by excessive ingestion of nonstructural carbohydrates such as starch or fructan. Sometimes no clinical developmental phase can be recognized, and the horse or pony is discovered in the acute phase of laminitis with no apparent ill health or inciting problem occurring beforehand. This appears to be the case with grass founder or laminitis resulting from the ingestion of pasture. Recently, Longland and Cairns (1998), researching the metabolism of grass in Great Britain, have shown that under certain climate conditions, fructan may reach very high concentrations in the stem of grass (<50% DM).

Fructan in Pasture

Trials on pasture consumption by horses in southeast Queensland (McMeniman, 2000) showed that during one warm, wet summer month pasture intake went from 5 kg/day to 15 kg/day. If fructan concentration during that month was 30%, then an intake of 3-4 kg fructan/horse/day was theoretically possible. However, none of the horses developed any health problems, so fructan accumulation was probably not occurring. If consumption of 3-4 kg of pasture fructan had occurred, laminitis was likely as dosing horses with 3-4 kg of pure commercial grade fructan (extracted from plants) causes experimental induction of laminitis almost without fail. The slower rate of intake of fructan from pasture may explain the difference between field intake from pasture and experimental boluses by stomach tube.

Horses and other herbivores in general are quite selective about what they eat, and there is a positive correlation between total nonstructural carbohydrate (TNC) content and plant selection. In other words, if one pasture species is in a sugar accumulation phase it will be sought out and consumed (Mayland et al., 2000).

Domestic horses encounter fertilized, irrigated, monocultured pastures across all seasons and may have little choice in what they consume. Under certain and yet ill-defined circumstances, fructans are produced by such grasses and are consumed by horses in high enough quantities to cause laminitis. Although starch is the major carbohydrate (CHO) contained in the seeds of pasture species, for much of the growing season it forms only a minor component of the total reserve of CHO within the plant. Prior to flowering, the leaves, stems, and tips of grasses accumulate a mixture of sucrose (glucose and fructose) and polymers of fructose (fructans), often to extremely high concentrations. Fructans function as reserve carbohydrates and are stored at different sites within the plant. A range of different internal and external factors influences patterns of fructan synthesis and storage. During flower development, fructan accumulation is high and can reach 50% of dry matter (Pollock and Cairns, 1991). Fructan concentration falls to insignificance when seed formation is complete and when drought and summer dieback occurs. With the advent of cooler, moister conditions, however, the formation of vegetative tillers occurs, and these are high in fructan. There is sound experimental evidence that chilling grass in the face of prolonged periods of radiant light stimulates a dramatic increase in fructan production (Pollock and Cairns, 1991). This is explained by photosynthesis driving sugar production (stored as fructan) while a reduction in the demand for carbon (less growth and metabolism) occurs because of chilling. Total tissue CHO content can reach 60-70% DM with up to 50% of this as fructan. It seems likely that horse pastures exposed to low nocturnal ground temperature and bright sunny days, as occurs in spring and autumn in most temperate regions, could accumulate dangerously high fructan levels, sufficient to trigger laminitis.

Pathophysiology of Laminitis

Inulin is a term applied to a heterogeneous blend of fructose polymers found widely distributed in nature as plant storage carbohydrates. Oligofructose is a subgroup of inulin, consisting of polymers with a degree of polymerization (DP) less than or equal to 10 (Niness, 1999). Inulin and oligofructose are fructans extracted on a commercial basis from the chicory root. Native chicory inulin has an average DP of 10 to 20, whereas oligofructose contains chains of DP of 2 to 10, with an average DP of 4 (Flamm et al., 2001). Inulin and oligofructose are not digested in the upper part of the gastrointestinal tract nor are they absorbed and metabolized in the glycolytic pathway or directly stored as glycogen. None of the molecules of fructose and glucose that form inulin and oligofructose appear in portal blood. They do not lead to a rise in serum glucose or stimulate insulin secretion. These materials are fermented by the microflora of the colon where they stimulate the growth of intestinal bifidobacteria. This fermentation produces D-lactate and a rapid drop in pH in the large intestine.

Enzymes capable of destroying key components of the hoof lamellar attachment apparatus (Pollitt and Daradka, 1998) have been isolated from normal lamellar tissues and in increased quantities from lamellar tissues affected by laminitis (Pollitt et al., 1998). The enzymes are metalloproteinase-2 and metalloproteinase-9 (MMP-2 and MMP-9). Lamellar tissues affected by laminitis upregulate their MMP gene (Kyaw-Tanner and Pollitt, unpublished), and increased amounts of MMP, in its active form, are found in laminitis affected tissues (Pollitt et al., 1998). Our evidence suggests that laminitis is triggered as increasing quantities of microbial substances manufactured in the large bowel from excess carbohydrate substrate are delivered to lamellar tissues via a vasodilated foot circulation (Pollitt and Davies, 1998). Testing a wide range of putative laminitis trigger factors (e.g., cytokines, eicosanoids, gram-negative bacterial endotoxins) with the *in vitro* laminitis model developed at the Australian Equine Laminitis Research Unit (AELRU), only the supernatants of cultured hindgut bacteria readily induced *in vitro* laminitis via the MMP activation pathway (Mungall et al., 2001).

Key pathologic changes include loss of cellular shape of the secondary epidermal lamellae and loss of attachment of the basement membrane of the lamellae, which is then lysed by MMP. The basement membrane is the key structure bridging the epidermis of the hoof to the connective tissue of the third phalanx, so it follows that the loss and disorganization of basement membrane leads to the failure of hoof anatomy seen in laminitis. Another component is loss of the lamellar capillaries, which may explain increased resistance to blood flow and the bounding digital pulse seen during early laminitis. The enzymatic theory of laminitis etiology challenges the existing view that laminitis develops from vascular changes to the circulation of the hoof. There is strong evidence that the foot circulation is vasodilated during the developmental phase of laminitis. This vasodilation is

important to allow a high enough concentration of trigger factors to reach the lamellar tissues.

Laminitis Induction Model

When consumed by horses, oligofructose is rapidly fermented in the hindgut. If the amount of oligofructose exceeds 7.5 g/kg live weight a gastrointestinal disturbance is triggered that somehow leads to laminitis. In the presence of excess oligofructose substrate, gram-positive microorganisms proliferate preferentially and become the dominant microflora. This altered population of hindgut microflora liberates substances that compromise the normally impervious epithelial mucosal barrier lining the lumen of the large bowel and causes it to leak. Permeability of the equine hindgut during the developmental stage of carbohydrate-induced laminitis has been demonstrated (Weiss et al., 1998).

In preliminary trials, we have induced mild laminitis by administering a bolus of commercially available fructan (Raftilose) into the stomach of horses. A dose of 7.5 g/kg results in laminitis 48 hours later, half the amount of starch required in the starch-induction model to induce laminitis. Because fructan is a soluble sugar, the bolus was easily dissolved in water and could be administered as a single dose via stomach tube. The animals developed a fever and projectile, acid diarrhea just as they do with the starch-induction model, but *without colic*. By 36 hours, the animals had normal feces, their appetite had returned, and they were returning to metabolic normality, but they had laminitis confirmed by histopathology (Pollitt, 1996). Higher doses of fructan led to more severe clinical laminitis. This is a new, more humane model for laminitis induction unique to our laboratory. At 24 hours, the feces were very acid and contain no gram-negative organisms and consisted of gram-positive rods and diplococci, thus supporting our contention that it is gram-positive organisms, rapidly proliferating on excess substrate, that are responsible for laminitis.

Cryotherapy as a Preventative Agent

Cryotherapy is an effective first aid strategy for a range of conditions, particularly in human medicine (Swenson et al., 1996). Anecdotal evidence suggests that it may be useful during the developmental phase of laminitis; several early texts recommend placing horses in cold streams to cool the feet. Indeed, digital vasoconstriction during the developmental stage appeared to protect horses against laminitis (Pollitt and Davies, 1998) and led Pollitt (1999) to suggest that cryotherapy may be an effective strategy to prevent laminitis. Support for the concept of laminitis cryotherapy came from the scintigraphic studies of Worster et al. (2000) that showed digital soft tissue perfusion was significantly reduced after the application of cold water for 30 minutes.

Six Standardbred horses were dosed with 10 g/kg oligofructose using the method described previously by Pollitt and van Eps (2002). Each horse had one front limb placed in a rubber boot containing a mixture of 50% cubed ice and 50% water for the duration of the 48-hour experimental period. The boot was continually replenished with ice to maintain a level just below the carpus. Clinical observations, including surface temperature of the hind feet, were made every two hours. Internal hoof temperature of the forefeet was monitored continuously with data-logging devices attached to temperature probes inserted 8 mm into the hoof wall on the dorsal midline. Internal boot temperature and ambient temperature were also monitored constantly with data-logging devices. All horses were euthanized at 48 hours, and stained sections of the hoof wall lamellae were examined with a light microscope. The severity of the laminitis was graded using the scoring system of Pollitt (1996).

Internal hoof temperature of the iced foot was maintained at less than 5° C at all times. During the last 12-15 hours of the experiment, hoof temperatures of the untreated feet exhibited a prolonged period of raised temperature consistent with digital vasodilation. All horses tolerated the ice boot well. Skin sensation and function of the iced limb was not impaired upon removal of the boot prior to euthanasia. All horses exhibited a degree of lameness associated with one or more of the untreated limbs. There was no significant histological evidence of laminitis in the treated hooves, but all of the untreated hooves had histological laminitis ranging from grade 1 (mild) to grade 3 (severe). Molecular biology performed on hoof tissue samples consistently showed normal expression of MMP2 RNA in the iced feet, with markedly increased expression in the nontreated feet.

Cryotherapy, when applied to one foot, was effective in preventing the development of acute laminitis in the face of a challenge that caused laminitis in the remaining three untreated feet ($p < 0.05$). We propose that vasoconstriction of the digital circulation during the developmental stage of acute laminitis prevents delivery of hematogenous laminitis trigger factors, probably of hindgut microbial origin (Mungall et al., 2001) to the lamellar tissue. The low temperatures achieved by the application of iced water to horses' distal limbs may also act to inhibit MMP enzymatic activity even if triggering factors were present. For ethical reasons, the laminitis induction experiments were terminated at 48 hours when the foot pain of laminitis appeared. What has not been determined by these experiments is whether laminitis could still develop after the period of cryotherapy had ceased. Cryotherapy is a potentially effective prophylactic strategy in horses with conditions placing them at risk of developing acute laminitis. By the time foot pain is apparent, lamellar pathology is underway, thereby missing an opportunity to prevent or ameliorate lamellar pathology.

Thus, we have discovered a pathway that links the metabolism of pasture grass (fructan production) to fermentative activity in the equine large bowel (gram-positive bacterial proliferation) that leads to laminitis (the triggering of uncontrolled

MMP activation and lamellar basement membrane destruction). Domestic horses encounter fertilized, irrigated, monocultured pastures across the seasons and have little choice in what they consume. Under certain, ill-defined circumstances, fructans are produced by such grasses and are consumed by horses in high enough quantities to cause laminitis. The task ahead is to devise ways of managing horse pastures to reduce fructan production, find a means of measuring fructan levels so dangerous pasture can be identified, find ways of managing horses to reduce pasture fructan consumption, and manage fructan-fermenting hindgut bacteria to prevent formation of laminitis trigger factors. Cryotherapy is a promising preventative agent in horses at risk of developing laminitis.

References

- Buffa, E.A., S.S. van den Berg, F.J.M. Verstraete, and N.G.N. Swart. 1992. Effect of dietary biotin supplement on equine hoof horn growth rate and hardness. *Equine Vet. J.* 24:472-474.
- Butler, K.D., Jr., and H. F. Hintz. 1977. Effect of level of feed intake and gelatin supplementation on growth and quality of hoofs of ponies. *J. Anim. Sci.* 44:257-261.
- Coenen, M., and S. Spitzlei. 1997. The composition of equine hoof horn with regard to its quality (hardness) and nutrient supply of horses. *Proc. 15th Equine Nutr. Physiol. Symp.* p. 209-212.
- Comben, N., R.J. Clark, and D.J.B. Sutherland. 1984. Clinical observations on the response of equine hoof defects to dietary supplementation with biotin. *Vet. Rec.* 115:642-645.
- Ekfalck, A., L.E. Appelgren, B. Funkquist, B. Jones, and N. Obel. 1990. Distribution of labelled cysteine and methionine in the matrix of the stratum medium of the wall and in the laminar layer of the equine hoof. *J. Vet Med. Series A* 37:481-491.
- Flamm G., W. Glinsmann, D. Kritchevsky, L. Prosky, and M. Roberfroid. 2001. Inulin and oligofructose as dietary fiber: A review of the evidence. *Critical Rev. Food Science and Nutrition.* 41:353-362.
- Geyer, H., and J. Schulze. 1994. The long-term influence of biotin supplementation on hoof horn quality in horses. *Schweiz. Arch. Tierheilkd.* 136:137-49.
- Goodspeed, J., J.P. Baker, H.J. Casada, and J.N. Walker. 1970. Effects of gelatin on hoof development in horses. *J. Anim. Sci.* 31:201 (abstr).
- Graham, P.M., E.A. Ott, J.H. Brendemuhl, and S.H. TenBroeck. 1994. The effect of supplemental lysine and threonine on growth and development of yearling horses. *J Anim. Sci.* 72:380-6.
- Hihami, A. 1999. Occurrence of white line disease in performance horses fed low-zinc and low-copper diets. *J. Equine Sci.* 10:1-5.
- Josseck, H., W. Zenker, and H. Geyer. 1995. Hoof horn abnormalities in Lipizzaner horses and the effect of dietary biotin on macroscopic aspects of hoof horn

- quality. *Equine Vet. J.* 27:175-182.
- Kempson, S.A. 1987. Scanning electron microscope observations of hoof horn from horses with brittle feet. *Vet. Rec.* 120:568.
- Lewis, L.D. 1995 In: *Equine Clinical Nutrition*. Williams and Wilkins, Philadelphia.
- Longland, A., and A. Cairns. 1998. Sugars in grass: An overview of sucrose and fructan accumulation in temperate grasses. In: *Proceedings of the Dodson and Horrell International Research Conference on Laminitis*. Stoneleigh, Warwickshire, England. pp 1-3.
- Mayland, H.F., G.E. Shewmaker, P.A. Harrison, and N.J. Chatterton. 2000. Nonstructural carbohydrates in tall fescue cultivars: Relationship to animal preference. *Agron. J.* 92:1203-1206.
- McMeniman, N.P. 2000. Nutrition of grazing broodmares, their foals and young horses. RIRDC publication 00/28. <http://www.rirdc.gov.au/reports/HOR/00-28.pdf>.
- Moore, J.M., D. Allen, and E.S. Clark. 1989. Pathophysiology of acute laminitis. In: *Vet. Clinics North Amer. Equine Practice* 5:67-72.
- Mungall, B.A., M. Kyaw-Tanner, and C.C. Pollitt. 2001. In vitro evidence for a bacterial pathogenesis of equine laminitis. *Vet. Microbial.* 79:209-223.
- Niness, K.R. . 1999. Inulin and oligofructose: What are they? *J. Nutr.* 129(7): 1402S1406S, Suppl. S.
- NRC. 1989. *Nutrient Requirements of Horses*, 5th Rev. Ed. National Academy Press, Washington, DC.
- Ott, E.A., and E.L. Johnson. 2001. Effect of trace mineral proteinates on growth and skeletal development in yearling horses. *J. Equine Vet. Sci.* 21:287-292.
- Ott, E.A., and E.L. Johnson. 2002. Nutritional factors influencing hoof development in horses. In: *Proc. Bluegrass Laminitis Symposium*.
- Pollitt, C.C. 1996. Basement membrane pathology: A feature of acute laminitis. *Equine Vet. J.* 28:38-46.
- Pollitt, C.C. 1999. Equine laminitis: Current concepts of inner hoof wall anatomy, physiology and pathophysiology. In: *Large Animal Proc. of the 9th Ann. Amer. College of Vet. Surgeons Symp.*, pp. 175-180. San Fransisco.
- Pollitt, C.C., and M. Daradka. 1998. Equine laminitis basement membrane pathology: Loss of type IV collagen, type VII collagen and laminin immunostaining. *The Equine Hoof. Equine Vet. J. Suppl.* 27:139-144.
- Pollitt, C.C., and Davies, C.L. 1998. Equine laminitis: Its development post alimentary carbohydrate overload coincides with increased sublamellar blood flow. *The Equine Hoof. Equine Vet. J. Suppl.* 26:125-132.
- Pollitt, C.C., M.A. Pass, and S. Pollitt. 1998. Batimastat (BB-94) inhibits matrix metalloproteinases of equine laminitis. *The Equine Hoof. Equine Vet. J. Suppl.* 26:119-124.
- Pollitt, C.C., and van Eps, A.W. 2002. Equine laminitis: A new induction model

- based on alimentary overload with fructan. In: Proc. Austr. Equine Vet. Assoc. Bain-Fallon Memorial Lectures (Abstr.).
- Pollock, C.J., and A.J. Cairns. 1991. Fructan metabolism in grasses and cereals. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:77-101.
- Reilly, J.D., D.F. Cottrell, R.J. Martin, and D.J. Cuddeford. 1998. Effect of supplementary dietary biotin on hoof growth and hoof growth rate in ponies: A controlled trial. *The Equine Hoof. Equine Vet. J. Suppl.* 26:51-57.
- Richardson, G.L., and E.A. Ott. 1977. Influence of protein source and lysine intake on growth and composition of hoofs of yearling foals. In: 69th Ann. Meeting Amer. Soc. Anim. Sci. p. 105 (Abstr.).
- Samata, T., and M. Matsuda. 1988. Studies on the amino acid compositions of the equine body hair and the hoof. *Jpn. J. Vet. Sci.* 50:333-340.
- Siciliano, P.D., K.D. Cully, T.E. Engle, and C.W. Smith. 2001. Effect of trace mineral source (inorganic vs organic) on hoof wall-growth rate, -hardness, and -tensile strength. In: Proc. 17th Equine Nutr. Physiol. Sym., p. 143-144.
- Swenson, C., L. Sward, J. Karlsson. 1996. Cryotherapy in sports medicine. *Scan. J. Med. Sci. Sports* 6:193-200.
- USDA. 2000. Lameness and Laminitis in US horses. National Animal Health Monitoring System. Fort Collins, CO. #N318.0400.
- Weiss, D.J., O.A. Evanson, J. MacLeay, and D. Brown. 1998. Transient alteration in intestinal permeability to technetium Tc99m diethylene triamino-pentaacetate during the prodromal stages of alimentary laminitis in horses. *Am. J. Vet. Res.* 59:1431-1433.
- Worster, A.A., E.M. Gaughan, J.J. Hoskinson, J. Sargeant, and J.H. Erb. 2000. Effects of external thermal manipulation on laminar temperature and perfusion scintigraphy of the equine digit. *N. Z. Vet. J.* 48:111-116.
- Zenker, W., H. Josseck, and H. Geyer. 1995. Histological and physical assessment of poor hoof horn quality in Lipizzaner horses and a therapeutic trial with biotin and a placebo. *Equine Vet. J.* 27:183-191.