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Laminitis - understanding the role of endocrinopathies and lush pastures

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Introduction

Laminitis is a common disease of horses which often causes severe pain and long-term debilitation. Management and treatment can be complicated, protracted and frustrating, leading to a poor outcome in moderate to severe cases. By understanding the causes of laminitis, a focus on prevention and effective early treatment can be made, resulting in an improvement in the overall health and wellbeing of equine patients.

The causes of laminitis can be categorised into four broad groups: endocrinopathic, supporting limb laminitis, repeated concussion/trauma, or conditions associated with systemic inflammatory response syndrome. The most common cause of the latter in adult horses is endotoxaemia, typically resulting from increased absorption of lipopolysaccharide (LPS) across the gastrointestinal tract (GIT), and thus laminitis is a risk for all horses with gastrointestinal disturbances.

Spring grass is often associated with laminitis, however it is important not to overlook predisposing or underlying factors.

Most cases of laminitis occur in horses and ponies kept at pasture; hence the oft used term "pasture associated laminitis".[1] The fact that two horses grazing the same pasture can have such different clinical outcomes (one laminitic, the other not), clearly highlights that factors other than diet are involved.

The interaction between the various risk factors for pasture associated laminitis is complex and not completely understood. For example, it has been demonstrated that over 90% of horses with laminitis have an underlying endocrinopathy, however the question remains whether in these cases the endocrinopathy is a primary or predisposing factor. The following technical bulletin will discuss pasture associated laminitis and its relationship with endocrine disease.

Section I: Pasture Associated Laminitis

The exact pathophysiology underlying pasture consumption and lamellar failure are not completely understood, but it is associated with horses grazing pastures that have high non-structural carbohydrate (NSC) content. In such animals the large quantities of carbohydrate delivered to the hindgut results in rapid fermentation and subsequently intestinal dysfunction as shown in **Figure 1**.

The systemic inflammatory response that occurs due to carbohydrate overload likely initiates local inflammatory and haemodynamic mechanisms which result in destruction of lamellar epithelium and extracellular matrix.

A number of risk factors are associated with development of pasture associated laminitis, including:

- Accumulation of high levels of NSC in pastures during spring, early summer or after heavy rain.
- Increased hours of daylight increasing nutritional intake of NSC.
- Concurrent endocrine disease (e.g. PPID, EMS).
- Phenotypic predisposition – "easy keepers", overweight or obese.
- Genetic predisposition – all pony breeds, "easy keeper breeds" (Egyptian Arabs, Spanish breeds, Appaloosa, Morgan, some lines of Warmbloods, Norwegian Fiord, Icelandic).[1,2]

Section II: Laminitis and Equine Endocrine Disease

Endocrinopathic laminitis describes cases of laminitis which occur in horses that have an underlying endocrinological disturbance. Most commonly, horses have either equine metabolic syndrome (EMS) or pituitary pars intermedia dysfunction (PPID). Less commonly, endocrinopathic laminitis has been associated with administration of exogenous corticosteroids for unrelated conditions.

Endocrine testing should be considered for all cases of laminitis as it has been recognised that 90% of laminitic horses will have an underlying endocrinopathy.

It has been recognised that up to 90% of horses presenting for laminitis have an underlying endocrinopathy, with 95% having phenotypic indicators of obesity.[6] This provides a basis for the understanding that endocrine testing should be considered in all cases of laminitis.

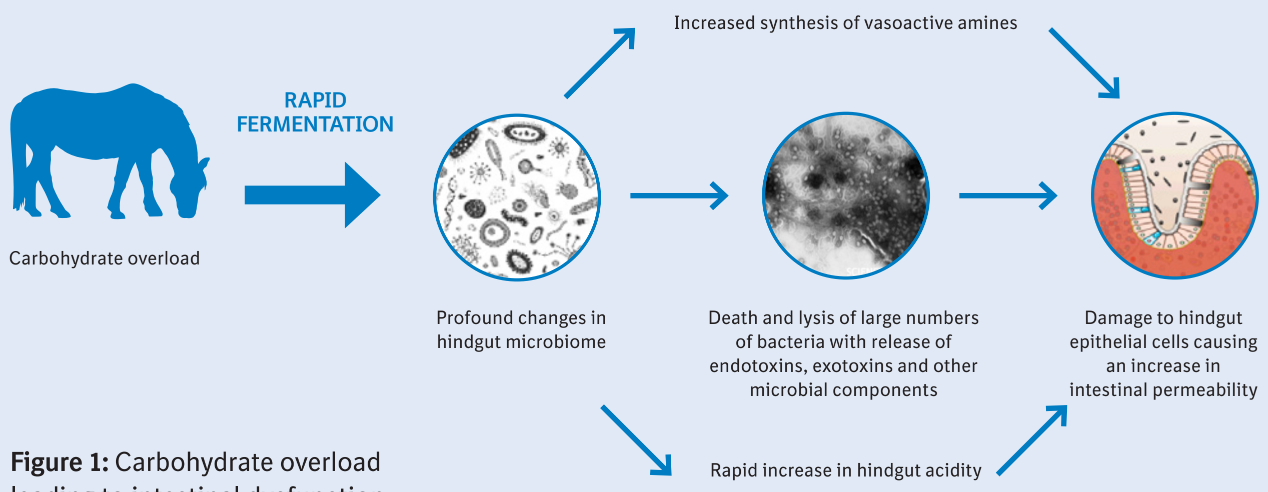


Figure 1: Carbohydrate overload leading to intestinal dysfunction

Results of extensive recent research has implicated insulin resistance (IR) and hyperinsulinaemia ('insulin dysregulation') as the critical abnormality responsible for the development of endocrinopathic laminitis in horses affected with both PPID and EMS.[7] It currently appears that lamellar dysfunction and injury is a direct effect of insulin on lamellar epithelial cells as opposed to lamellar energy deprivation.[7] Aberrant growth factor signalling and decreases in lamellar concentrations of the activated form of AMP-activated protein kinase (AMPK) may play a pivotal role in altering control of cytoskeletal and cell adhesion dynamics, resulting in disruption of lamellar epithelial cytoskeletal dynamics.

The evidence is currently lacking for a direct cause and effect relationship between elevated corticosteroids and laminitis as adrenocortical hyperplasia is an inconsistent finding and plasma cortisol levels are often normal in PPID-affected horses.[7] Therefore, in horses with PPID, it appears that laminitis is a consequence of existing/coexisting EMS or insulin dysregulation as the result of the combined actions of melanocortins and hypercortisolism (if present). Inhibited inflammatory responsiveness represents a very important clinical component of PPID because signs of pain associated with endocrinopathic laminitis may be inhibited leading to severe and detrimental disruption of the digital lamellae in physically active PPID-affected individuals due to uninhibited mechanical loading.[5]

Section III: Diagnosis of Equine Endocrine Disease

In suspected cases of endocrinopathic laminitis, diagnostic testing should be performed for both EMS and PPID. Testing is widely available for both disease states and is relatively easy to conduct.

No seasonal patterns for insulin or glucose have been identified, but there is a distinct circannual rhythm for ACTH secretion in horses which is linked to day length (**Figure 2**).[8] In Australia, there is a quiescent phase between June and November, as day length is increasing, where ACTH levels are lower with little variability. This is followed by a dynamic phase, as day length decreases, where ACTH levels are higher and more variable. A peak in ACTH levels appears to occur at the autumn equinox when the decrease in day length from day to day is at its greatest. Individual reference intervals have been recommended to account for this.

Measurement of resting ACTH for the diagnosis of PPID is recommended in the dynamic phase as PPID horses experience an exponentially larger increase in ACTH at this time of the year compared to normal horses.

Diagnostic testing for PPID is recommended during the dynamic phase, from February to April.

Figure 3 provides a general guide for a clinical testing protocol with interpretation of results. As there is a large body of research being currently undertaken, all reference intervals are subject to future refinement. Specific values may be allocated by age, gender, breed, time of year and location as research progresses.

However, thyrotropin releasing hormone (TRH) stimulation testing is currently only recommended during the quiescent phase as responses to TRH are highly variable in the dynamic phase.

Equine ACTH Levels - Annual Rhythm

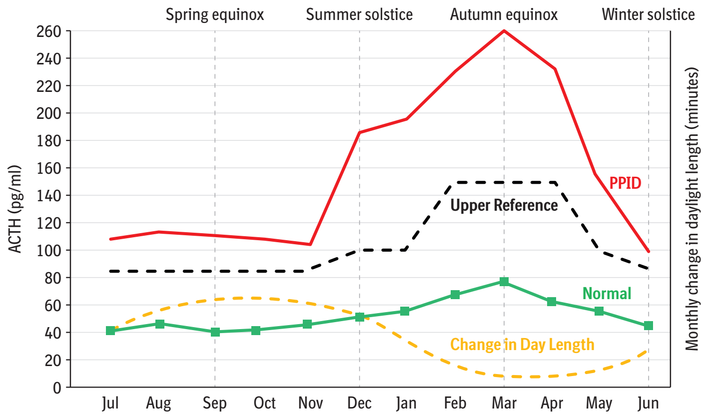


Figure 2: Representative ACTH levels expected in normal and PPID horses relating to change in day length.

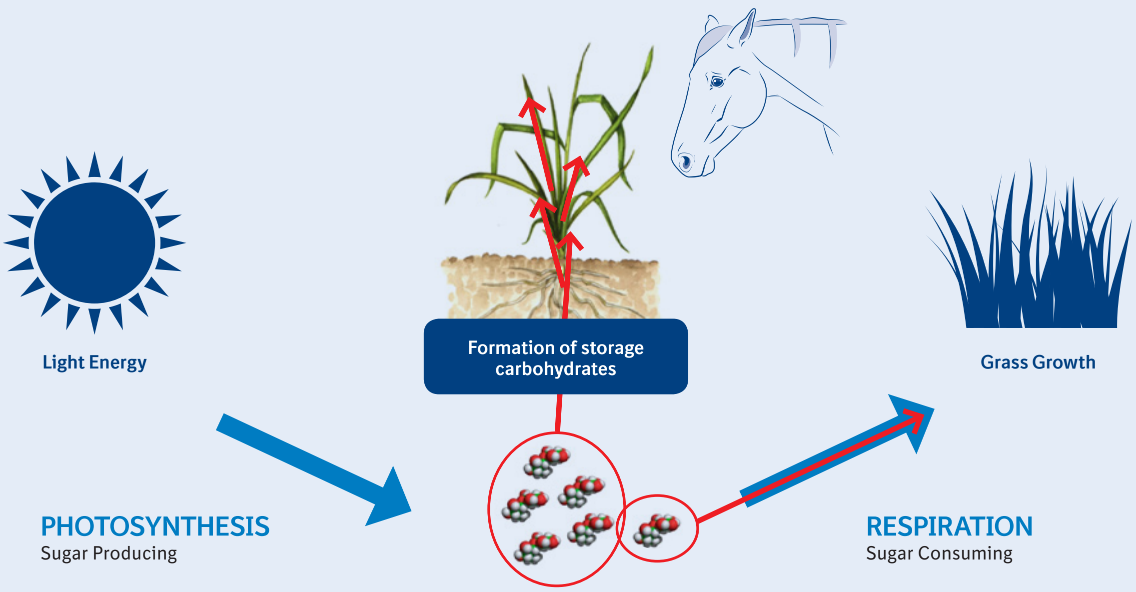
Why is spring grass a problem?

Understanding grasses and their NSC content

Horses are selective grazers that can spend up to 60 to 70% of their time grazing if given free access to pasture. As much as 75% of the dry matter of forage consists of structural and non-structural carbohydrates (NSC). Structural carbohydrates are indigestible to horses but are of nutritional value via microbial fermentation in the large colon.[1] Simple sugars, starches, oligosaccharides and soluble fibres comprise the NSC component. **The amount of NSC within a plant can vary depending on a number of variables including the plant species, the environment, and the stage of growth.** We can divide plants into either C3 or C4 species, with the terminology relating to the number of carbon atoms that make up the first product of photosynthetic metabolism in these species. C3 species, typical of Australian native grasses, preferentially accumulate fructan as the primary storage carbohydrate. C3 grasses are also referred to as temperate or cool season grasses. C4 grasses, also known as tropical or warm season grasses, preferentially accumulate starch as a storage carbohydrate.[3,4]

At its most basic the concentration of NSC in plants represents a balance between the sugar producing process of photosynthesis, and the sugar consuming process of respiration. Sugar accumulation occurs when it is created in excess of what is required for growth, which triggers the formation of storage carbohydrates. Understanding the factors that influence these two processes can therefore provide insight into the likely NSC content of plants, and therefore the risk they may pose to susceptible grazing horses. Understanding this also explains why spring grasses present the greatest problem.

Photosynthesis utilises light energy to create simple sugars and is active whenever there is light and the plants are not frozen or dessicated.[4] C4 grasses have more efficient photosynthesis under hot conditions and this metabolic process is not saturated until the plant is in full sunlight.[4] In contrast photosynthetic efficiency of C3 grasses



Excess sugar formulation leading to storage of carbohydrates within the grass peak at half the amount of luminescence.[4] Therefore, in hot, dry days with full sun and long daylength, C4 grasses have double the photosynthetic capacity of C3 plants compared to cool, wet, cloudy conditions in which C3 grasses have an advantage.[4] Regardless of class, both types of grasses have a risk of causing laminitis due to overload of soluble sugars.

The sugar produced through photosynthesis is utilised in the process of respiration, whereby the plant creates energy for growth and maintenance. There are a range of environmental stresses that can hinder growth, leading to an accumulation sugars and subsequently NSC. The enzymes responsible for respiration are temperature dependent. At low temperatures (< 5°C for C3 plants and < 10-15°C for C4 grasses) respiration and therefore growth ceases, leading to accumulation of NSC. This effect is greatest when the temperature fluctuates in and out of the cold range. In spring, this factor, along with increased photosynthetic activity associated with longer day lengths, results in high NSC concentrations. In summer, with the absence of respiration ceasing cold temperatures, NSC are generally low, while in autumn, with less daylight for photosynthesis, the concentrations are intermediate. In addition to cold temperature, other stresses such as heat stress, drought stress, and nutrient deficiencies can impair respiration and lead to accumulation of NSC.

In C3 grasses, NSC concentration is higher in stems than leaves and increases in concentrations towards the base of the plant. In C4 grasses, excess NSC is stored as starch in leaf tissue and seed heads. Developing seed heads become the primary NSC sink and at flower stage, peak NSC concentration is present throughout the plant.[3]

Quality pastures are essential in the provision of energy and nutrient requirements for horses. However, widespread pasture improvement selecting for grasses with high NSC content has meant that horses are often grazing pastures primarily meant for production animals, particularly cattle, which increases the danger of carbohydrate overload.

ENDOCRINE EVALUATION

Equine Metabolic Syndrome (EMS)

OR

Resting Insulin

Protocol:
Fast for 3 hours or access only to low NSC/soaked hay (rather than grazing/grain) in the prior 4 hours.

Result	Interpretation[a]*
<20 µU/ml	Normal, Non diagnostic**
20-50 µU/ml	Suspect insulin dysregulation
>50 µU/ml	Insulin dysregulation

Comments:

*Low sensitivity but high specificity test i.e. EMS positive horses may have normal resting insulin but EMS negative horses are unlikely to have high insulin.

**Dynamic tests (e.g. OST) are more sensitive and should be considered as a primary test and if resting insulin measurement is low despite concurrent signs supportive of EMS.

Oral Sugar Test (OST)

Protocol:
Fast horse overnight (12h). Feed 0.5 to 1 g/kg of glucose or dextrose mixed in a small amount of non-glycaemic feed e.g. chaff. Collect blood 2h after feeding for insulin measurement.

Insulin dysregulation if:
>68 µU/ml (0.5g/kg OST)
>85 µU/ml (1g/kg OST)

Pituitary Pars Intermedia Dysfunction (PPID)

Resting ACTH

Protocol: Sampling at any time of day for ACTH (pg/ml) [b#]

Interpretation	Jun-Nov	Dec, Jan, May	Feb-Apr[#]
Negative	<50	<75	<100
Re-test	50-85	75-100	100-150
PPID Positive	>85	>100	>150

Comments: [#]Testing is recommended during the dynamic period when PPID horses have markedly elevated ACTH levels in comparison to normal horses. Isolated spurious elevations may occur, particularly during the dynamic period – repeat resting ACTH in 2-4 weeks to confirm results in horses with minimal concurrent signs or horses that are undergoing stress or other painful disease processes.

TRH stimulation testing has a higher diagnostic accuracy and should be considered in those horses requiring re-testing.

TRH Stimulation Test

Protocol: Blood collection for ACTH prior to, and at 10 or 30 mins after administration of 0.5-1 mg IV per horse of TRH during the quiescent period. Do not feed grain or perform OST prior to test.

PPID positive if ACTH >100 pg/ml at 10 mins or >65 pg/ml at 30 mins[a].

Comments: TRH responses are highly variable in the dynamic period and reference ranges have yet to be established.

[a] *Equine Endocrinology Group, 2015 and 2016 Consensus Recommendations, http://sites.tufts.edu/equineendogroup*

[b] *Australia and New Zealand Equine Endocrinology Group, 2017 Consensus Recommendations*

Figure 3: Clinical testing protocol for diagnosing EMS and PPID

Section IV: Safe Spring Grazing - Avoiding Pasture Associated Laminitis

Restricted grazing

Sugars accumulate during daylight through the process of photosynthesis, and as the day progresses, peaks in the later afternoon to early evening. Susceptible horses and ponies may need to be completely withheld from green grass pastures during spring due to the longer day length with concurrent cold nights resulting in high NSC accumulation caused by reduced night time utilisation through respiration. The same is true regarding sunny days in which there has been frost overnight. After several nights of warm weather and good growing conditions, access to pasture can be re-assessed as NSC levels are reduced by morning. Restricted grazing may then be considered in the early morning with 1-2 hours used as a starting point.

Grazing muzzles can be used for horses that can tolerate some grazing. Close observation is warranted to ensure that the horse adapts to wearing one and that limited intake is actually achieved, as some horses are very adept at successfully grazing large quantities of pasture despite its use.

If there has been a period of drought, when rain occurs, restrict or avoid grazing new grass until there at least 2 leaves per tiller on the new growth. As horses graze the first green shoots, they often ingest a large amount of stubble and crown material that have high concentrations of NSC, stored under drought conditions, which are yet to be utilised for growth.

Managing pasture to minimise NSC concentration

Native grasses are often lower in NSC but do not withstand heavy grazing except on large acreage with few horses and are inherently less winter hardy.[3] Therefore, without land management and rotational grazing techniques, replanting paddocks with lower NSC grasses in an intensive grazing situation is generally not an option. Instead, focus is on managing existing pastures to minimise NSC concentration.

The following management practices can reduce the NSC content in pastures:[4,5]

- Avoid paddocks with varieties of grass developed for weight gain in production animals.
- Avoid over-grazing which stresses pasture and increases the NSC concentration in surviving stems.
- Pastures shaded by buildings or strategic tree planting will have lower NSC content associated with restricted sunlight exposure.
- Rotational grazing allows grasses, particularly lower NSC grass species, periods of recovery to "R&R" (rest and regrow). This allows sustainable pastures and discourages more aggressive weed growth.
- Control weeds as some, such as dandelion, wild oats and quackgrass, are palatable to horses and very high in NSC.
- Strategic mowing prior to development of seed heads will redirect grass resources into growing more tillers resulting in a higher leaf to stem ratio which spreads NSC concentration more evenly throughout the plant.
- Soil should be well fertilised and irrigated to allow optimum growth to avoid stresses which can increase NSC levels.

Pasture alternatives

If horses require complete removal or restricted access to pastures, alternative feed sources will be required to fulfil nutritional requirements. Ideally, hay with a low NSC content (<12%) should be selected as the basis for the diet.[5] Be aware of the following when selecting hay:[4,5]

- Sugar content of hay does not decrease during storage.
- Hay with a large amount of stem can be very high in NSC.
- The environmental conditions and stage of growth at the time of baling significantly effects NSC content.
- Colour of hay has no relationship to sugar content i.e. brown hay may have equal or higher NSC content than green hay.
- Exact NSC composition of hay cannot be determined without forage analysis and will vary between batches.

If the NSC content of hay is unknown or suspected to be high, soaking can be used to reduce sugar concentration. General recommendations for soaking hay are:[4,5]

- Concentration of water soluble carbohydrates (sugar and fructan) can be reduced by soaking in water for 1-2 hours.
- Extended soaking is not recommended as other water-soluble nutrients are probably also leached out.
- Water should be discarded prior to feeding as the sugars will remain.
- Starches are not soluble in water and forages containing high starch levels will be unaffected by soaking.
- Feed soaked hay within 12 hours to avoid mould growth, particularly in warm weather.
- Broad spectrum vitamin and mineral supplements should be provided.

Low NSC-feedstuffs in small, more frequent meals per day can be provided if additional calories are required and include:[5]

- Highly digestible fibre sources such as sugar beet pulp (soaked with water removed and without added molasses) or soya hulls.
- Low NSC, high-fibre, high-oil commercial feeds.
- Addition of vegetable oil rather than cereal starch as an energy source.

Section V: Management of Equine Metabolic Syndrome (EMS) and Pituitary Pars Intermedia Dysfunction (PPID)

Horses with evidence of insulin dysregulation should be maintained on a low glycaemic diet and fed for weight loss if obese. Daily exercise is also recommended, but not for those horses with active laminitis. Medical therapy with levothyroxine (0.1 mg/kg PO, daily) is indicated for those horses which are obese and require accelerated management or have resistance to weight loss. Metformin hydrochloride (15-30 mg/kg PO, 1-3 times daily) can also be used alone, or in conjunction with levothyroxine, when there is poor compliance with dietary recommendations or persistent hyperinsulinaemia.

PPID affected horses should have a good preventative health program and often benefit from hair coat clipping to ameliorate the effects of hypertrichosis. Pergolide (Prascend®) is the recognised treatment for PPID at an initial dosage of 0.5 mg for a 250 kg pony and 1.0 mg for a 500 kg horse (2 µg/kg) with the dose administered once daily. Dosage may need to be increased during the dynamic period if clinical signs worsen.

Section VI: Summary

Laminitis is a painful and debilitating disease of horses. It is important to identify animal risk factors and diagnose concurrent endocrine disease to implement effective medical and dietary management. This is particularly vital during spring, when cases of pasture associated laminitis are at their highest.

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References: 1. Goer RJ. Current concepts on the pathophysiology of pasture-associated laminitis. *Vet Clin North Am Equine Pract* 2010;26(2),265-276. **2.** Watts K. Pasture management to minimise the risk of equine laminitis. *Vet Clin North Am Equine Pract* 2010;26(2),361-369. **3.** Watts KA. Forage and pasture management for laminitic horses. *Clin Tech Eq Pract* 2004;3(1),88-95. **4.** Watts KA, Pollitt CC. Equine Laminitis - Managing pasture to reduce the risk. Rural Industries Research and Development Corporation, 2010. **5.** Belknap JK, Goer RJ. Equine Laminitis. Wiley-Blackwell, 2017. **6.** Karikoski NP, Horn I, McGowan TW et al. The prevalence of endocrinopathic laminitis among horses presented for laminitis at a first-opinion/referral equine hospital. *Dom An End* 2011;41(3),111-117. **7.** Frank, N. and Tadros, E.M. (2014) Insulin dysregulation. Equine Vet. J., 46 (1), 103–112. **8.** Secombe CJ, Tan RHH, Perrara DI, et al. The Effect of Geographic Location on Circannual Adrenocorticotropic Hormone Plasma Concentrations in Horses in Australia. *JVIM* 2017 Jun 29 doi: 10.1111/jvim.14782