Building practical strategies against mycotoxins

Dr. Ruth Bishop  
Technical Director, MARS Horsecare UK

Every horse feed is comprised of the nutrients and ingredients that it’s design requires, but it will also contain unavoidable undesirable substances. Control of these is an essential part of compiling a safe and wholesome diet.

Such “undesirables” can be chemical (e.g. heavy metals), physical (e.g. blister beetles) or microbiological, such as moulds (e.g. *Aspergillus fumigatus*), and bacteria (e.g. *Salmonella* spp.). Mycotoxins, chemical substances produced by certain moulds under stress conditions are chemical undesirable substances.

Moulds are present on all agricultural products, and production of mycotoxins from moulds can be triggered during critical parts of plant growth cycles by stresses such as drought, crop damage and adverse weather during maturation. High quality harvested crops can be similarly compromised by poor bulk storage and handling.

It is well known that at certain levels mycotoxins can affect animal health and performance. Those mycotoxins currently considered to be of relevance to the equine are listed in Table 1.

Table 1: Mycotoxins currently considered to be of potential relevance in equine diets and the main moulds that produce them.

<table>
<thead>
<tr>
<th>Mycotoxin (when typically formed)</th>
<th>Mould *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ochratoxin A (formed in field and during storage in temperate regions)</td>
<td><em>Penicillium verrucosum</em></td>
</tr>
<tr>
<td>Ochratoxin A (formed during storage in tropical regions)</td>
<td><em>Aspergillus ochraceus</em></td>
</tr>
<tr>
<td>Zearalenone (mainly formed in the field but also during harvesting and storage)</td>
<td><em>Fusarium spp.</em></td>
</tr>
<tr>
<td>Deoxynivalenol &amp; Nivalenol (mainly formed in the field but also during harvesting and storage)</td>
<td><em>Fusarium graminearum, F culmorum</em></td>
</tr>
<tr>
<td>T2 and HT2 (mainly formed in the field but also during harvesting and storage)</td>
<td><em>Fusarium langsethiae, F poae, F sporotrichoides</em></td>
</tr>
<tr>
<td>Fumonisins (grow on senescent or stressed plants)</td>
<td><em>Fusarium moniliforme (verticilloides)</em></td>
</tr>
</tbody>
</table>
Aflatoxin (formed in field and during storage)  Aspergillus flavus

Lolitrem B (formed in the field)  Neotyphodium lolii

Ergot alkaloids (formed in the field)  Neotyphodium spp.

Slaframine (formed in the field)  Rhizoctonia leguminicola

Swainsonine (formed in the field)  Swainsonia spp.

* Most relevant species producing that mycotoxin

Whilst mycotoxins are undesirable, they can be present in the feedstuffs and forages that horses eat as a logical consequence of the natural appearance of moulds, so that supplying universally mycotoxin-free feed is not a realistic expectation. However by understanding and applying safe limits, we can ensure that the presence of a mycotoxin does not automatically define a feed as toxic.

Currently, incomplete information exists with respect to the safe limits (e.g. NOAEL, LOAEL and PSI) for mycotoxins in equine diets or feedstuffs. However there is effective guidance for maximum levels in feedstuffs in legislation (for Zearalenone, Deoxynivalenol, Ochratoxin, Fumonisin and Aflatoxin). In addition, for T2 and HT2 Toxins, the European Food Safety Authority (EFSA) recently published a Scientific Opinion which concluded that a Benchmark Dose derived for pigs could also be applied as a reference point for equines.

By operating within such limits, the equine feed industry can provide the horse with feed that is safe according to current regulatory guidance and opinion.

Given avoidance of mycotoxins is difficult, and horses do not appear to self-protect by automatically refusing mycotoxin-contaminated feeds, principal mitigation against mycotoxins is therefore focused on reducing where possible their presence in feed ingredients. This is especially important as there is no routine procedure available for detoxification, and normal feed processing does not eliminate mycotoxins.

Mitigating (or risk management) activities can take place at all stages of the feed chain as illustrated below:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>Management of the crop production chain (through for example: selection of varieties, ploughing, disposing of the residues of former crops, sequence of crops, use of fungicides, initiation of insect repelling programmes, limiting infection pressure from natural reservoirs, machinery maintenance.)</td>
</tr>
<tr>
<td>Harvest</td>
<td>Conditions at harvest, Grain drying to &gt;13ºC, cleaning of dried grains. For</td>
</tr>
</tbody>
</table>
forages, avoid harvesting dead plant material or soil; wrapping haylages securely in thick polythene (small bales) or 6-8 layers of plastic (large bales)

<table>
<thead>
<tr>
<th>Storage</th>
<th>Protection against damage in the storage unit. Low moisture environment, controlled free water activity, insect and mite defence. Additionally certain preservatives used in store (e.g. propionic acid) may prevent mould growth.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Good manufacturing practice and HACCP</td>
</tr>
</tbody>
</table>
| Distributor of finished goods | Good finished product storage and stock management (e.g. First in, first out stock rotation)  
(Ingress of moisture from leaks or vermin damage can cause mould growth and potentially lead to mycotoxin formation.) |
| End user | Correct storage and adherence to sell by dates. |

The potential inhibition of mycotoxin uptake by the animal via in-feed mycotoxin binders has been embraced in other species but requires more definition in the horse. Currently, mycotoxin control in horses is available in the form of feed materials, as there are no mycotoxin-binding additives currently authorised for use in equines in the EU. Feed materials used for mycotoxin control in horses are based on yeast cell wall products. However, ultimately much more data is needed to scientifically validate the use of binders in the horse.
Rethinking equine stereotyping: A neurological perspective: What the horse brain is telling us

Dr. Sebastian McBride
Research Associate, University of Cambridge

Repetitive, invariant behaviours known as stereotypies are observed in several farm, companion and captive animal species including the horse (Mason and Rushen, 2006). Equine stereotypies include crib-biting, weaving and box-walking and exist within 7-9% of the population (McGreevy et al., 1995). These behaviours develop as a result of chronic stress exposure associated with specific events such as weaning (Nicol et al., 2005), as well as general husbandry that restricts feeding, reduces social contact, and diminishes locomotor activity (McBride and Hemmings, 2009). More recent research has identified the underlying neurophysiological mechanism (brain region and neurotransmitter system) of equine stereotypy development. Specifically it is considered that dopamine modulation of the basal ganglia brain region is critical to the onset of these behaviours (McBride and Hemmings, 2005). This part of the brain is primarily responsible for learning and motivation, and when it becomes dysfunctional in humans, the clinical conditions of Parkinsons and Huntingtons disease.

This paper presents the brain pathway involved and discusses whether having this additional insight into the underlying mechanism potentially provides additional strategies of prevention and attenuation of these types of anomalous equine behaviour.

Utilizing nutrition to manage equine genetic disorders

Dr. Stephanie Valberg
Professor and Director of the Equine Center, University of Minnesota Equine Center

Equine practice has recently embraced nutrigenomics, in which a horse’s nutritional requirements are tailored to its individual genetic make-up. Equine nutrigenomics has been applied to the dietary management of genetic disorders such as polysaccharide storage myopathy (PSSM), recurrent exertional rhabdomyolysis (RER) and hyperkalemic periodic paralysis (HYPP). Diets with elevated vitamin E are also recommended for families of horses producing foals with equine degenerative myeloencephalopathy (EDM) and neuraxonal dystrophy (NAD). This talk will focus on how dietary management of the above mentioned disorders can successfully prevent clinical signs in genetically susceptible individuals.
Prevention of obesity: potential role of the hindgut microflora in horses

Dr. Lucy Waldron
President and Founder, LWT Animal Nutrition

Twenty years ago the role of hind gut micro-organisms was scarcely taken into account in non-ruminants in relation to their nutrition and related disorders. Since then, the role of the hind gut microflora has been of increasing interest and subject to much research, starting with agricultural species such as pigs and chickens, and now being applied to pets and horses. From the equine side, the main focus on the characterisation of hind gut microflora has centred around its role in laminitis (Milinovich et al., 2005; 2007; 2008a, b), and the ‘triggers’ from feed and/or microbes that release bioactive amines that cause inflammation and laminitis symptoms (Elliot and Bailey, 2006). However there are other aspects that are also of importance that have been revealed from studies with other mammals, and which need to be considered in horses.

For the purpose of this paper, we will consider the potential influence of hind gut bacterial populations on weight gain in horses. Obesity is a major issue for many horses and ponies, and, whilst being strongly linked to laminitis via insulin resistance among other aspects (Respondek et al., 2011), it also poses other major health risks to the animal. Studies have been conducted in other mammalian species, including mice, chickens and dogs, where the bacterial profiles of the gut have been elucidated via genetic techniques (Raoult, 2008; Bermingham, 2009; Apajalahti, 2012). These have shown that the microbial profile of an obese animal is significantly different from a non-obese animal. So can we use these findings to extrapolate to horses? As all equine nutritionists and horse owners know, there are the so-called ‘good doers’ and ‘poor doers’ within the horse population. These sub-groups cannot be directly related to breed (although some breeds are associated with obesity more than others) or management strategies. For example, obese thoroughbred and arab horses do occur, even if they are managed and fed in the same way as their more common ‘poor doer’ counterparts. Research published by Koike et al. (2000) compared the microflora of native Japanese ponies against thoroughbreds and found that the cellulytic Fibrobacter succinogenes was more dominant in the ‘good doer’ ponies compared to the lighter breed horses. So, leaving aside issues such as laminitis, is there a perfect microbial profile for a horse? Is it possible to influence the microbial profile by nutritional intervention or the application of specialist feed ingredients to stabilise or promote an optimal microflora that will ensure that horses are neither too fat or too thin? We already know that feeding pre or probiotics or yeast products to animals can be beneficial, but are normally used within the context of fattening up an underweight horse. Work conducted with oligosaccharides has shown that major feed changes, e.g. a high barley diet, showed that horses fed a prebiotic had fewer changes in microbial population than the control (Respondek et al., 2008; de Fombelle et al., 2001). Additionally, the application of Lactobacillus based probiotics can reduce foal scouring by up to 40% (Lawlor, 2011). In a review by Julliand (2005), it is again highlighted that the mode of action of both pre and probiotics in horses is not well characterised, which needs addressing via research. However the amount of information on bacterial types and levels is improving.

So, these are some indications out there in the published research – but what can we do to tie this together to give a clearer picture on combating obesity, with all its associated disease problems, in a practical manner? Controlled studies are needed, where the bacterial profiling of fat, thin and ‘normal’ horses are obtained over a range of feeds (e.g. varying from 100% forage to high concentrate). Once these profiles have been obtained, further work has to be done applying feeding strategies and supplements, including pre and probiotics, yeast preparations and so on, to observe any shifts in population towards the ‘normal’ profile. With suitable funding, it should not be impossible to formulate
a feeding program that will optimise the horse’s hind gut environment and minimise gastric and obesity issues in future.

References
Lawlor, C. (2011) Online article The Horse Magazine, Australia
Strengthening antioxidant status in endurance horses

Dr. Carey A. Williams
Associate Professor, Rutgers, The State University of New Jersey

Supplementing antioxidants such as vitamin E, vitamin C, and lipoic acid appears to benefit endurance horses by decreasing oxidative stress and muscle enzyme leakage and by increasing antioxidant status. Although many investigators have examined equine response to exercise and supplementation in terms of lipid peroxidation, antioxidant status, and other biomarkers, much remains to be learned. Meanwhile, caution must be taken when supplementing antioxidants above recommended levels because of potential interference with the absorption of other nutrients. Research highlights from studies of antioxidant supplementation in endurance horses follow.

Vitamin E (alpha-tocopherol) is the most commonly supplemented antioxidant in horses. In competing endurance horses, vitamin E intake was calculated to be 1150 to 4700 IU/d, which is 1.2 to 5 times higher than levels recommended by NRC (2007). Even though vitamin E intake negatively correlates with muscle enzymes (creatine kinase and aspartate aminotransferase), indicating lower levels of muscle membrane leakage with higher vitamin E intake, caution should be taken when supplementing with high levels of vitamin E. We have investigated pharmaceutical levels of vitamin E for impact on oxidative stress, muscle enzymes and antioxidant status. Horses supplemented with vitamin E at nearly 10 times the NRC (2007) level did not experience lower oxidative stress but were found to have lower plasma beta-carotene concentrations, which may indicate that vitamin E has an inhibitory effect on beta-carotene metabolism.

Previous work has shown that vitamin C potentiates the effects of vitamin E by reducing the tocopheroxyl radicals and restoring their activity. Under maintenance conditions, horses have the ability to synthesize sufficient ascorbate, but demand increases as stress increases. We looked at the vitamin E and C interaction in endurance horses supplemented with vitamin E alone or in combination with vitamin C for three weeks before a race. We found no oxidative stress or antioxidant status differences between these supplements with the exception of a higher concentration of plasma ascorbate before, during, and after racing. These results are similar to those found in competing polo ponies using a similar supplementation protocol.

Using the novel antioxidant, lipoic acid, we found that apoptosis of white blood cells was 40% lower in Arabians trained to run on an equine treadmill compared with the WBC apoptosis rate in unsupplemented horses. Plasma alpha-tocopherol and ascorbate also increased, as well as red blood cell glutathione, which could have aided in scavenging radicals, thus triggering the apoptosis. Other recent studies have examined the effects of superoxide dismutase on oxidative stress and inflammation in exercising horses systemically and locally (synovial fluid) with little evidence of benefit.
Stallions in AI (artificial insemination): Management, housing, infection risks and nutritional influences on semen quality

Dr. Karsten Zech
Manager of Horse and Cattle Service, Chamber of Agriculture, Lower Saxony Chamber

The European Community Council directive 92/65/EEC is the basis for the approval of EC semen collection centers. These centers must be under the permanent supervision of an officially authorized center veterinarian. Centers should be readily cleaned and disinfected, and it should be constructed so that contact with other animals or unauthorized people is prevented. Conditions required for the approval of EC-semen collection centers include a lockable animal accommodation (stallion stable) with a separate exercise area, semen collection facilities with slip-proof flooring, a separate room for disinfecting equipment, a semen processing room, isolation facilities, and a semen storage room.

Prerequisites for the use of stallions in AI: Stallions must be free of clinical signs of infectious diseases at the time of admission to the center and the days of semen collection. Thirty days before the dates of semen collection, stallions should be kept in holdings without clinical signs of equine viral arteritis or contagious equine metritis, and they should not be used for natural mating.

For fresh semen production: Donor stallion quarantine at the beginning of the breeding season is required. Testing requirements to control the spread of infectious diseases are detailed in ECC directive 92/65/ECC.

Feeding of AI-stallions: During the breeding season heavily used stallions have additional needs for energy and protein as well as amino acid composition. Omega-3-fatty acid-enriched diets seem to have no effect in normal stallions in regard to spermatozoal membrane integrity, motility, viability, but have possible effects on semen freezability in stallions of marginal fertility.

Trace elements: An adequate selenium supply is associated with improved equine sperm quality and fertility. Researchers have shown correlations ($P \leq 0.05$) between Se levels in spermatozoa (nmol/billion) and progressive motility ($r = 0.31$), membrane integrity ($r = 0.40$) and acrosomal status ($r = -0.42$) as well as correlations ($P < 0.03$) between Se concentration in spermatozoa (nmol/g) and pregnancy rate per oestrus cycle ($r = 0.40$). Zinc in spermatozoa and seminal fluid have been shown to correlate negatively with age, DNA fragmentation index, and $\alpha$-t-value as a parameter for chromatin integrity.