Moulds and mycotoxins in animal feeds:

implications for equine health and performance.

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1.1 Introduction

A horse's diet consists mostly of grass or grass products, such as hay or silage. As complementary, additional feed, different types of grain or cereal are often given, either directly as a mixed grain feed, or processed into pellets. Bedding is used in a horse's stall, most often in the form of straw, which consists of dried stalks of cereal plants, such as wheat. Moulds (fungi imperfecti) can be found growing in or on grasses, cereals and grains, both during the growth of the plant (pre-harvest) and after harvest, during storage. Moulds can produce toxic substances called mycotoxins. These mycotoxins can cause intoxications when they are found in the feed or bedding of animals. There are many different kinds of mycotoxins, produced by different moulds. Mycotoxins can have different adverse health effects on horses. They can affect the athletic performance of horses and influence the reproductive system. Even subclinical intoxications can reduce the economic value of a horse, since horses are mostly used for riding or breeding. Finally, horses are true companions for many of their owners and impairment of the welfare and performance of a horse results in emotional damage.

The knowledge about potential adverse effects of moulds and mycotoxins is incomplete as detailed investigations have only incidentally been conducted. The present review aims to summarize the most important facts relating to moulds and mycotoxins as undesirable contaminants of feed materials commonly used in the diet of horses.

1.2 Mould invasion before harvest

Moulds can invade plants before harvest. They may be phytotoxic as in the case of Fusarium species, which impair plant growth and harvest yields. Toxin production occurs already in the living plants and may contribute after harvest during storage and ensiling under favorable conditions. They are commonly known as Fusarium ear rot on corn ears.

Figure 1: Fusarium ear rot, fungal growth on corn ears.
http://www.ent.iastate.edu/imagegal/plantpath/corn/fusarium/fusarium_insect_inj.html

In contrast to these phytopathogenic moulds, the large group of endophytic moulds (endophytes) are representative for a beneficial symbiosis between fungi and a living plant. Endophytes improve the resistance of plants against viral, bacterial and insect damage, and may induce the production of anti-stress factors conferring resistance of plants against heat stress and draught.

Endophytic moulds live in a true symbiotic relationship with their host plants and usually enter the seeds of their host (Bourke 2009). They protect the seeds against parasites and helminths, while at the same time use seed as a vector for their own dissemination. However, they can also grow in other parts of the plant, as shown in figure 2. Endophytes are mostly found in grasses and related monocotyledons, Typical endophytic species occurring in forage grasses are *Neotyphodium lolii and N, coenophialum,* while *Rhizoctonia leguminicola* invades particularly legumes

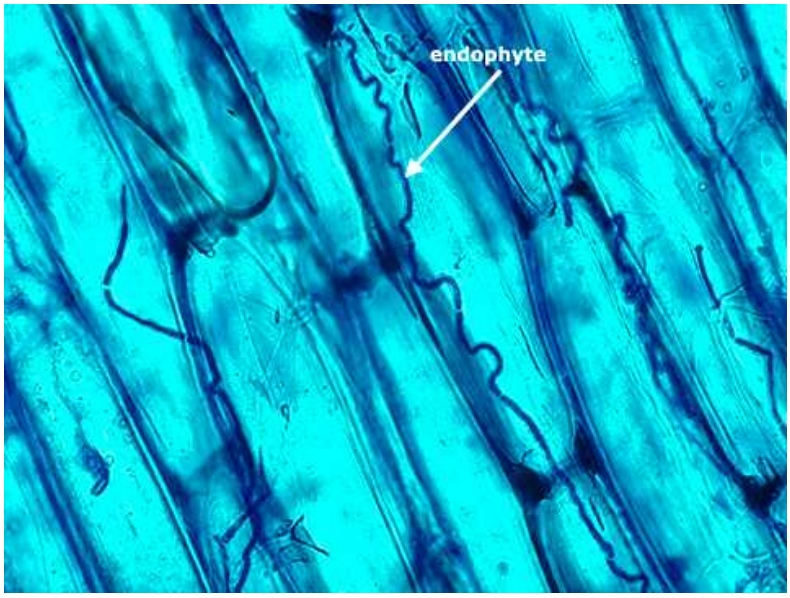


Figure 2: The tall fescue endophyte (*Neotyphodium coenophialum*) in the tiller. Photo by Nicholas Hill (Roberts and Andrae 2004).

1.3 Mould invasion after harvest

Grasses can be stored as hay or grass silage and grains are processed into mixed feeds or pellets, which can all be stored for many months. One of the main problems of storing horse feed is the threat of infection by toxicogenic moulds, such as *Aspergillus* and *Penicillium* species, known to invade plants or plant products after harvest and produce mycotoxins. These moulds flourish in moist conditions, which occur when packaging is not done properly or storage facilities are not suitable. This is the case when packaging is not airtight and when water(droplets) is able to get to the feed. In modern agricultural practice, grains are dried post harvest to a moisture content of less than 14 %, to achieve microbiological stability. Incomplete drying and re-moistering during storage are the main risk factors for grains and cereals, while in silage undesirable high residual amounts of oxygen (incomplete compaction) favor mould growth in this intermediate moisture materials. Taking better precautions when storing horse feed can prevent such a secondary contamination with moulds and mycotoxins.

1.4 Routes of Exposure

Horses can be exposed to mycotoxins by various routes of exposure. The most obvious route of exposure is through their feed. Horses are herbivores and their diet consists mostly out of hay or grass and pellets or grains. Moulds can be found in both grasses and grains and can

therefore be eaten by the horse, which allows the mycotoxins to be taken up by the digestive system. Since horses do not have a fore-stomach system there is no pre-systemic reduction of the toxin exposure.

Another way for horses to come into contact with mycotoxins is through their bedding materials. Usually straw is used in a horse's stall, see figure 3, this is made of the dried stalks of cereal plants, such as wheat and barley. Mycotoxins in straw can affect the horse when ingested and by direct contact with the skin or through inhalation of fungal spores. Sometimes other types of bedding materials are used, such as wood shavings or pellets and even material like moss and paper. All of these materials are based on plant matter and could be invaded by moulds in various degrees. Straw is still used most often as bedding material, probably due to economic reasons and because it is readily available. When considering the effects of different moulds and their mycotoxins it is important to keep in mind through which route of exposure the horse is exposed.

Figure 3: A foal sleeping on a bed of straw. (Photo by Lisa Spronck)

Obviously, in exposure assessment the amount of mycotoxin a horse is exposed to also has to be determined. Table 1 shows an overview of the concentration of mycotoxins found in horse feed. This shows many different mycotoxins, produced by different fungi. It is clear that fumonisin B, DON and zearalenone are studied more intensively than the other mycotoxins.

Table 1.

Concentrations of toxins produced by moulds, found in equine feeds.

Toxin	Concentration in ppm	Source
N-acetyl norloline	200 - 2000	Bourke 2006
Peramine	6 – 25,9	Bourke 2006
Fumonisin	0,7	Reyes-Vasquez
Fumonisin	5	Scudamore 1997
Fumonisin B1	28,5	Rosiles 1998
Fumonisin B1	1- 3,8	Scudamore 2009
Fumonisin B1	7,49	Keller 2007
Fumonisin B1	0,11	Sacchi 2009
Fumonsin B1	5,1	Monbaliu 2010
Fumonisin B2	0,18 - 1,2	Scudamore 2009
Fumonisin B3	0,13 - 0,71	Scudamore 2009
Lolitrem B	1,73 - 5,15	Johnstone 2011
Lolitrem B	0,97 - 2,22	Miyazaki 2001
Ergovaline	0,52 - 1,17	Miyazaki 2001
Zearalenone	0,39	Monbaliu 2010
Zearalenone	0,8	Raymond 2005
Zearalenone	0,034 – 0,07	Skladanka 2011
Zearalenone	0,04 – 0,16	Scudamore 2009
Zearalenone	0,48	Reyes-Vasquez
Zearalenone	0,1	Raymond 2003
Zearalenone	0,5	Scudamore 1997
Deoxynivalenol	0,22 – 0,93	Scudamore 2009
Deoxynivalenol	0,7 – 1,2	Raymond 2003

Deoxynivalenol	6,7	Reyes-Vasquez
Deoxynivalenol	11,2	Raymond 2005
Deoxynivalenol	0,033 – 0,046	Skladanka 2011
Deoxynivalenol	9,53	Monbaliu 2010
Deoxynivalenol	1,23	Wichert 2008
Aflatoxin B1	0,1	Keller 2007
Aflatoxin	0,016	Reyes-Vasquez
Aflatoxin	0,014	Gunsen 2002
Aflatoxin	0,041	Scudamore 1997
fusaric acid	3,9 – 12,3	Raymond 2003
fusaric acid	40,5	Raymond 2005
15-ADON	0,7	Raymond 2005
Ergosterol	35,65 – 82,53	Skladanka 2011
Ochratoxin	3,66 – 38,4	Pozzo 2010
Ochratoxin	0,006	Reyes-Vasquez
Ochratoxin A	0,033	Monbaliu 2010
Ochratoxin A	0,1	Scudamore 1997
Citrinin	0,008	Scudamore 1997

2.1 Endophytic moulds

Endophytic moulds are moulds that invade plants to undergo a mutualistic symbiotic relationship. They grow inside a plant and can be spread through the seeds of the plant. One of the main endophytic genera are *Neothyphodium* species, previously known as *Acremonium.* Endophytic moulds only grow in living plants, but their mycotoxins can be found in processed horse feed. Horses are often exposed to endophyte mycotoxins when the horses are grazing in dry pastures or following the consumption of hay. Endophytic moulds can be very useful in areas where draught and/or infections threaten pastures. The mould can offer protection of for example grass as is shown in figure 4: the side infected with an endophyte is more resistant to insect damage (beetles) than the side without an endophyte infection. This shows why endophyte infected grass is often seen as positive. This grass grows faster and looks better than grass that is not infected. The beneficial effects of endophytes are often caused defined secondary metabolites exerting an anti-parasitic activity. At the same time, and often by related synthetic pathways, endophytes produce substances (mycotoxins) that are toxic for mammalian species, including horses. There are several different mycotoxins produced by endophytes that cause disease in horses. Most of these diseases are named after the plant that hosts the endophytic mould, like Perennial Ryegrass Staggers disease (PRGS) of Fescue foot (Festuca specis as host plants).

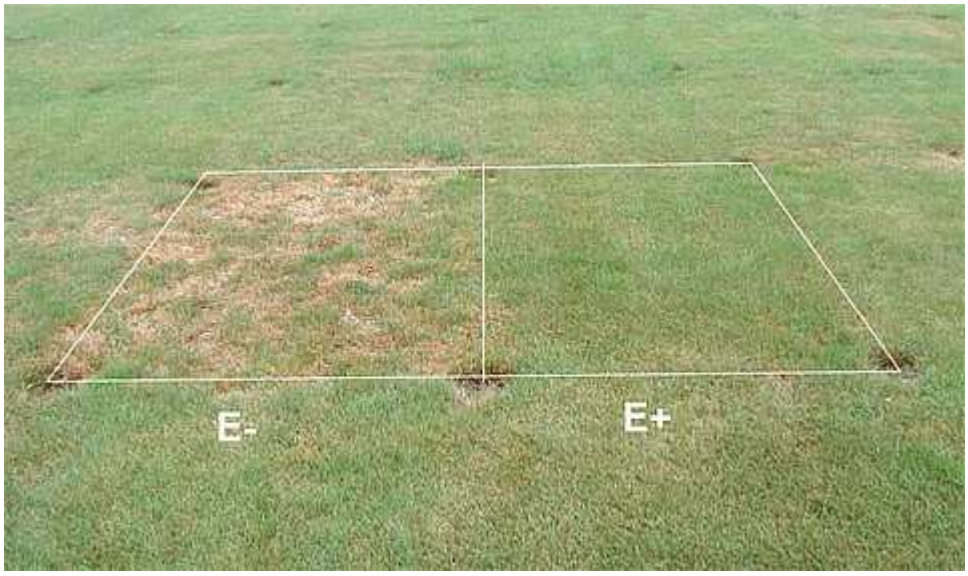


Figure 4: resistance of endophyte infected grass (E+) against Red thread caused by *Laetisaria fuciformis.* The endophyte-free grass (E-) shows less growth and is more affected than the endophyte infected grass. http://www.plantmanagementnetwork.org/pub/ats/research/2005/endophyte/

2.2 Tall Fescue

Tall Fescue (*Festuca arundinacea*) is a perennial pasture grass that is often infected with *Neotyphodium coenophialum,* an endophyte, which contributes to the health of the plant by acting as an insecticide. To provide this, the fungus produces different toxic ergot alkaloids, such as ergovaline (Panaccione 2005), but also other groups of alkaloids like peramines and lolines (Bush 1997). The toxicity of ergovaline had been established in livestock, but the other two toxins have not been investigated thoroughly (Bourke 2009). Endophyte infected tall fescue can cause Equine Fescue Toxicosis, which is mostly found in the USA. The effect of tall fescue on horses is different than the effect tall fescue has on other animals. The body temperature of horses does not increase after exposure to the endophytes toxins, unlike cattle (Rhodes 1991). This may be due to the fact that horses sweat more easily than cattle (Putnam 1991). In cattle a well-known effect of peripheral vasoconstriction caused by the toxins is 'Fescue Foot', which causes a gangrenous condition of the feet, see figure 5 (Solomons 1989). This has not been reported in horses (Cross 1995). Clinical signs of fescue toxicosis in pregnant mares include prolonged gestation, abortion, weakness, dysmaturity and mortality, thickened or retained placentas and attenuated lactation or agalactia (Cross 1995, Brendemuehl 1997, Green 1997).

Figure 5: Fescue foot on a cow (Roberts 2004).

Ergopeptine alkaloids are thought to stimulate D2-dopamine receptors. This stimulation results in decreased secretion of prolactin (hypoprolactinemia) (Cross 1995). This hormone is involved in the endocrine regulation of lactogenesis and can therefore affect milk production. Hypoprolactinemia could also be responsible for prolonged gestation by altering the in utero feto-placental steroid metabolism (Cross 1995, Evans 2002). Due to the negative effects on the reproductive system removal of pregnant mares from the endophyte-infected pasture has

been recommended to be done 60 to 90 days before the expected foaling date to prevent these symptoms (Evans 2002). This will give the body time to recover from a possible mycotoxin infection before the foal is born.

2.3 Perennial Ryegrass

Eating perennial rye grass (*Lolium perenne*) infected with *Neotyphodium lolii* or hay prepared from this grass can intoxicate horses. This fungus produces the mycotoxin Lolitrem B, as most toxic substance in a range of related secondary fungal metabolites.. This toxin causes a neurotoxic effect, commonly known as perennial ryegrass staggers (PRGS), in sheep, cattle, deer, alpaca and horses (Mackintosh 1982, Holmes 1999, Fink-Gremmels 2005). Lolitrem B is easily absorbed and can be found in high concentrations in fatty tissue (Latorre 2006). The toxin has been found to bind to calcium-activated K+ channels (BK channels) thereby inhibiting K+ currents exiting the cells (Dalziel 2005, Imlach 2011). Symptoms of lolitrem B intoxication include tremor, muscle fasciculation, ataxia, truncal swaying and tetany, which increase by stress or exercise (Tor-Agbidye 2001, Fink-Gremmels 2005). The level of lolitrem B in horse plasma did not correlate to the severity of the tremors in an *in vivo* study in which horses were exposed to lolitrem B (Johnstone 2011), suggesting that accumulating tissues concentrations are responsible for the described adverse effect.

There is no known effective treatment against Lolitrem B intoxications, but when it is diagnosed correctly, lolitrem B intoxication is completely reversible and once removed from the infected feed animals generally recover within 5 days (Blythe 2007). The only deadly outcomes reported from staggers disease were animals so disoriented that they walked into bodies of water and were unable to get out. The problem in diagnosing this disease is that intoxication with lolitrem B causes symptoms that can be confused with other neuro-degenerative diseases.

The endophytic mould grows mostly at the base of the grass and in the reproductive parts at the top of the stalks. So to keep mould growth to a minimum the grass should not be overgrazed, but not be allowed to grow too long either to prevent flowering, a physical stimulus that favors mycotoxin production. When the grass is kept at the right length, exposure to lolitrem B can be reduced, which will thereby reduce the risk of intoxication.

2.4 Legumes

Toxicosis by exposure to endophytes can also result from eating legumes. *Rhizoctonia leguminicola* is an endophytic mould, which can be found in red clover (*Trifolium pretense)* and other clover species (Hibbard 1995). The fungus produces two indolizidine alkaloids, slaframine (l-acetoxy-6-aminooctohydroindolizine) and swainsonine (1,2,8-trihydroxyocta-hydroindolizine) (Wickwire 1990, Croom 1995, Fink-Gremmels 2005). Both of these compounds are synthetized from L-lysine, see figure 6 (Broquist 1985). Slaframine causes excessive salivation, which gives it the name Slobbers disease. Another name is black patch disease, after the black spots the fungus makes on the leaves of legumes. Excessive salivation can be the only clinical sign found (Wijnberg 2009), but longer exposure to slaframine can result in anorexia, bloating, diarrhea, urination and lacrimation and even death (Hibbard 1995, Fink-Gremmels 2005). However, clinical signs may cease within 48 hours after removal from contaminated pasture (Wijnberg 2009).

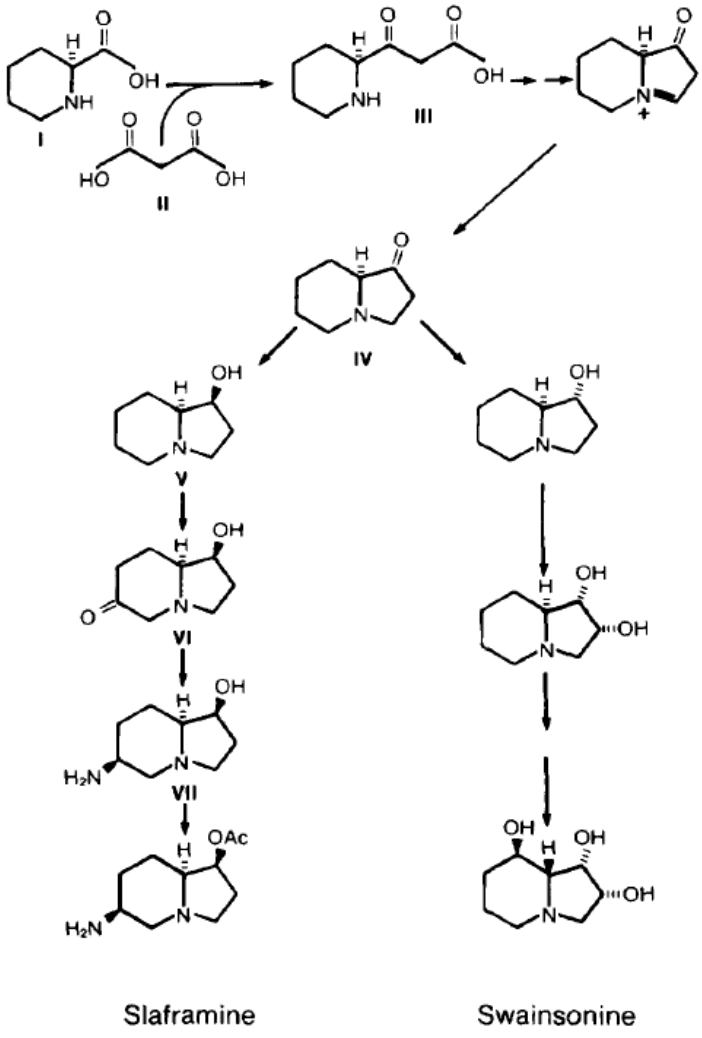


Figure 6: metabolism and structure of slaframine and swainsonine from L-lysine. (Broquist 1985)

Slaframine intoxication can be treated with atropine, which is an acetylcholine receptor antagonist (Croom 1995). This is effective because slaframine toxicity is caused by the structural similarity of keroimime, a metabolite of slaframine, to acetyl choline, which acts on muscarinic receptors (Wijnberg 2009). When not all clinical signs of slobbers disease are blocked by using atropine swainsonine is usually also affecting the horse (Fink-Gremmels 2005). Swainsonine has different effects than Slaframine, such as a staggering gait, depression, muscular incoordination, appetite suppression, staring, and nervousness (Croom 1995). Swainsonine inhibits Golgi α-mannosidase II, which is an enzyme that is involved in N-linked glycosylation (van den Elsen 2001). It is clear that legumes infected with moulds can pose a serious threat to horses, with potentially severe consequences. However, when diagnosed in time, there are some treatment options, and horses can recover from intoxication.

3.1 *Fusarium* Toxins

Fusarium species are often found on corn (maize) plants and can produce a variety of toxins, which are likely the most prevalent on a global basis (Wood 1992). These moulds are not beneficial to plants, in contrast to the endophytes discussed before. Most of the Fusarium mycotoxins are produced in the pre-harvest phase, but may sometimes be produced after harvest as well, on the surface or in oxygen containing cavities of the silage. Mycotoxin production is influenced by humidity and temperature and can vary between source and season (Scudamore 2009). The three major toxins produced by *Fusarium* species are Fumonisins, Zearalenone and Trichothecenes (Placinta 1999). When a mixture of these toxins is found, this usually means that there are different moulds present. Raymond et al demonstrated the effect of feeding *Fusarium* mycotoxins to horses (Raymond 2003, 2005). Horses were fed hay and concentrates that contained a combination of deoxynivalenol (DON), 15-acetyldeoxynivalenol (both trichothecenes) and zearalenone (ZEA). The effects that were found were appetite suppression and weight loss. New fusarium toxins are still being discovered, all potentially dangerous to horses (Scott 2012)

3.2 Fumonisin B

A major group of mycotoxins produced by *Fusarium* species are the fumonisines. Fumonisin B1 (FB1) is the most common fumonisin, exceeding FB2 concentrations by three times and by twelve times for FB3 (Sydenham 1991). The structures of these three fumonisines are shown in figure 7. The most well known fumonisin is fumonisin B1 (FB1) which causes the neurologic condition described as Equine Leukoencephalomalacia (ELEM) (Marasas 1988, Plumlee 1994). This has also been shown experimentally in different studies, either by injecting FB1 toxin (Foreman 2004) or by feeding horses feed contaminated with the toxin (Wilson 1992). FB1 has different effects on other animal species, such as nephrotoxicity and carcinogenicity in rats, and pulmonary edema in pigs. It seems though, that horses are the most sensitive species (Caloni 2010). Early signs of ELEM are paralysis of the tongue, forelimb placing deficits and hind limb ataxia, which may occur hours or days before severe clinical signs (Foreman 2004). Due to the neurologic nature of the symptoms it can be difficult to diagnose. Characteristic signs of ELEM are frenzy, incoordination, blindness, aimless circling, paresis, ataxia, head pressing, depression and hyperexcitation (Ross 1991).

Mortality due to ELEM is high and death can occur within hours or days from onset of the clinical signs, or even without clinical signs (Caloni 2010).

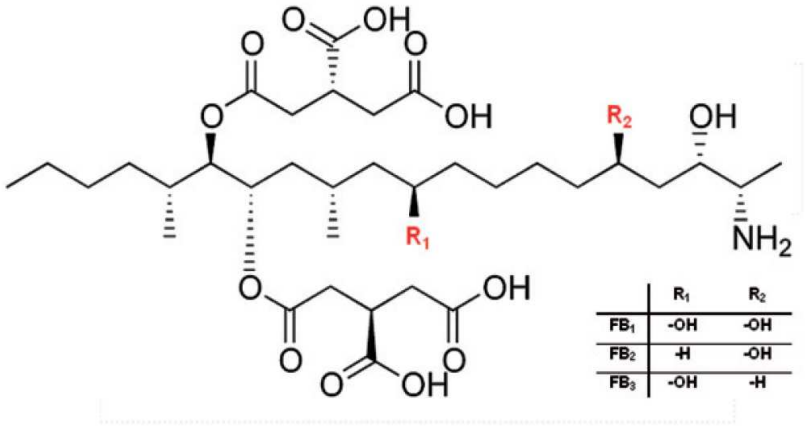


Figure 7: Structure of Fumonisine (B1, B2 and B3) (Scott 2012).

A study of 45 confirmed ELEM cases showed that most of these horses had been exposed to >10ppm of FB1. This suggests that a concentration of FB1 in feed >10mg/kg is not safe for horses, compared to 160 ppm in pigs, which shows that horses are more sensitive (Ross 1991). FB1 is thought to occupy the space of fatty acyl-CoA in ceramide synthase, this inhibits the enzyme from being acylated (Merrill 1996). This binding can be reversed by removal of FB1, so the inhibition is not permanent. The effect of Fumonisin B has also been studied *in vitro* (Minervini 2010a). The problem with fumonisin toxicity is that it is so sudden. Even if the correct diagnosis is made soon after the first symptoms of ELEM are found, it will probably still be too late to save the horse. Removal of the infected feed will have no effect because the disease will have advanced too far.

3.3 Zearalenone

Zearalenone (ZEA) is a mycoestrogen, which is a mycotoxin that resembles an estrogen, that can be found on cereals and grains worldwide (Fink-Gremmels 2008). The chemical conformation of ZEA is non-steroidal but still resembles 17β-estradiol and therefore it can bind to estrogen receptors, the structure is shown in figure 8 (Songsermsakul 2006). So the estrogen receptor is activated by exposure to ZEA. This causes hyperestrogenism (enlarged

uteri and nipples, vulvovaginitis, ovarian cysts) and impaired fertility (Diekman and Green 1992). It is mainly produced by *F. graminearum* and is completely different from trichothecenes in structure. Pigs are considered to be the most sensitive among farm animals. Studies performed on pigs show that the effect ZEA had depends on the reproductive status of the animal. Studies done to examine the effect of ZEA on horses are almost exclusively *in vitro*. ZEA was shown to induce cellular disturbances in granulose cells isolated from equine ovaries (Minervini 2006). The same group demonstrated that ZEA and its derivatives α-ZOL and β-ZOL affected equine sperm chromatin structure (Minervini 2010b). Studies have shown that α-ZOL shows the highest binding affinity to estrogen receptors, followed by ZEA itself (Shier 2001). β-ZOL shows the relatively lowest binding affinity to estrogen receptors (found in rodents). This indicates that the reaction of an animal to ZEA depends on the metabolism of the compound. Equine hepatocytes have been shown to favor the metabolism into α-ZOL above β-ZOL. This indicates that horses could be sensitive to ZEA intoxication.

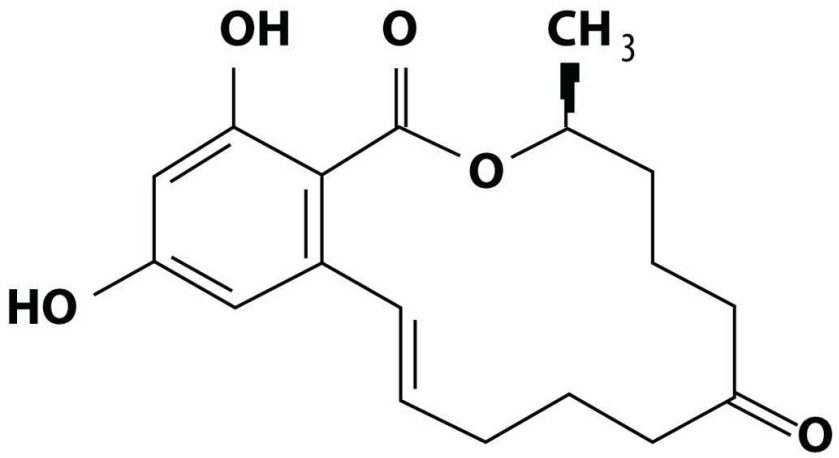


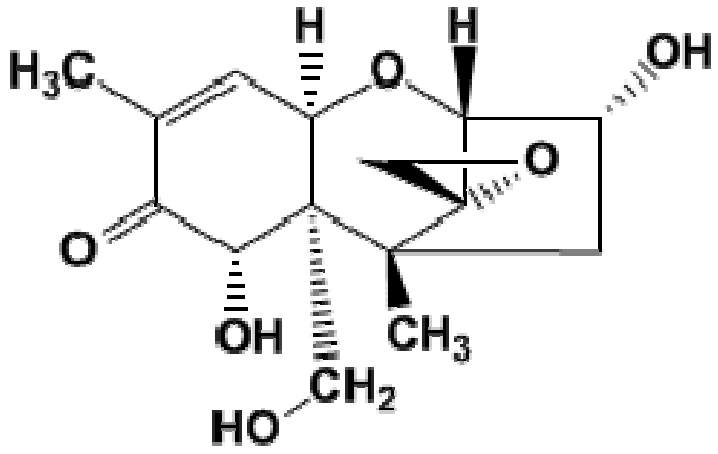
Figure 8: Structure formula of zearalenone (Banjerdpongchai 2010)

Filannino *et al* found that equine sperm motility was only affected by α-ZOL, not by ZEA or β-ZOL, which supports the idea of α-ZOL being more toxic than the parent compound and the other metabolite (Filannino 2011). *In vitro* studies were also performed using equine granulosa cells (GCs) which showed that GCs are especially sensitive to the effects of α-ZOL and ZEA (Minervini 2006). These data suggest a role of ZEA in equine reproduction. The effects of ZEA could be a problem in horse breeding. Breeding quality horses is a costly

business and if the reproductive system of breeding mares is affected by ZEA the chances of getting them with foal could be reduced.

3.4 Trichothecenes

Another group of mycotoxins are the trichothecenes. The main source of trichothecenes is cereal grains, like all fusariotoxins. There are many different tricothecenes like deoxynivalenol (DON), shown in figure 9, nivalenol, T-2 toxin and diacetoxyscirpenol (DAS) (Placinta 1999). Some trichothecenes are known as feed refusal toxins because they can induce loss of appetite (Rotter 1994). Information on the effect trichothecenes have on horses is scarce (Caloni 2010). Deoxynivalenol (DON) is a trichothecene that interacts with the dopaminergic system of the central nervous system, which can induce nausea and vomiting and is therefore also known as vomitoxin (Pestka 2004). The effect of DON on horses has been studied but has shown contrasting outcomes. Johnson *et al* showed no adverse effects after exposure to 36-44 ppm of DON. (Johnson 1997). Another group of horses exposed to feed that was naturally contaminated with *Fusarium* mycotoxins was shown to have a loss of appetite and weight loss (Raymond 2003, 2005). This diet consisted of a mixture of toxins (DON, fusaric acid, zearalenone, and 15-acetyldeoxynivalenol) at lower concentrations than the study done by Johnson et al, which suggests possible synergistic effects of the toxins (Caloni 2010). There is little information published on the other trichothecenes on horses. There is a report in which six mares were fed T-2 *Fusarium* mycotoxin during summer and early autumn. The only effect that was found were oral lesions in three of the mares (Juhasz 1997). T-2 toxin is less commonly found in feed than other trichothecenes, like DON, but it is thought to be more potent (Caloni 2010).



www.biosite.dk 140200

Figure 9: Structure formula of Deoxynivalenol (DON), a trichothecene (www.biosite.dk).

4.1 Storage Moulds and their toxins

The moulds and mycotoxins previously described are all found in or on the plant before harvest, although the mycotoxins production does not always cease after harvest. Another group of moulds are the storage moulds. These moulds usually infect feeds post-harvest, but can occasionally be found pre-harvest. The moulds are often found on hay, silage or grains that have been exposed to moisture or oxygen, mostly due to poor packaging or storage conditions. It is important to store roughage and grains properly to prevent mould growth.

There are two species of mould that are predominantly found in silage; *Penicillium roqueforti* and *Aspergillus fumigatus*. The first being the predominant species in European and Asian studies and the latter is the predominant species in North American studies (Fink-Gremmels 2005). Both of these species of mould can produce mycotoxins under certain circumstances. These mycotoxins can affect horses negatively. To prevent intoxication, all feed infected with mould should be removed from the horses. Mould can grow inside the packages of feed unseen, but it is often visible when the feed is opened, see figure 10. This can alarm horse owners and will most likely stop them from feeding it to their horses. However, parts of the feed can look unaffected by the mould, while still containing mycotoxins. So horses can still be exposed to mycotoxins, without the horse owner knowing about it.

4.2 Aflatoxin

Aflatoxin is a mycotoxin mainly produced by *Apergillus* species and is found on peanuts, soybeans, maize, and among other foods. The production of Aflatoxin is highest when the temperature is around 25 to 30 degrees Celsius with a humidity of almost 100% (Norholt 1976). Aflatoxins affect liver functions and induce centrilobular necrosis, which results in loss of feed intake, weight loss and reduced productivity (Stoloff and Trucksess 1979, Fink-Gremmels 2008). Other symptoms of aflatoxin intoxication can be depression, fever, tremor, ataxia and cough (Larsson 2003). These effects can greatly influence the results of horses used for their athletic capabilities. An effect aflatoxin can have after long-term low mycotoxin exposure is impairment of the immune system. This may cause increased susceptibility to

infectious diseases and lower immune response to vaccination programs (Fink-Gremmels 2008). Aflatoxin is also known for its carcinogenic effects and is found to be the most potent hepatocarcinogen (WHO 1993). A total dietary concentration of 500–1000 ug/kg (ppb) has been shown to induce clinical changes and liver damage, depending on the duration of exposure (Meerdink 2002). There may also be a link between aflatoxin exposure and COPD, which will be discussed later (Caloni 2011).

Figure 10: Mould growth on a recently opened bale of chopped maize.
http://www.ilvo.vlaanderen.be/tenv/NL/Onderzoek/Voedselveiligheid/Microbiologisch/Beheersing_ongewenste_schimmels_kuilvoeder/tabid/663/Default.aspx

4.3 Ochratoxins and Citrinin

In contrast to Aflatoxin, which affects the liver, the target organs of Ochratoxins are the kidneys. Ochratoxin is produced by *Penicillium* and *Aspergillus* species. Ochratoxin intoxication show mild signs, such as an initial increase in water uptake and excretion. Mortality is usually low, but just as with Aflatoxins the immune system can be affected, which reduces the health status of the horse. There is not much known about the toxicity of Ochratoxin on horses as not many studies have been performed (Duarte 2011). Ochratoxins are thought to affect the enzymes that are involved in phenylalanine metabolism. Just like Ochratoxin, Citrinin is produced by both *Penicillium* and *Aspergillus* species. In all species that the mycotoxin was tested it showed nephrotoxic effects, but not much is known about the toxicity in horses (Flajs 2009).

Penicillium roqueforti is a storage mould that, besides the mycotoxins already mentioned, produces toxins such as Roquefortine (mainly Roquefortine C) and Mycophenolic acid. This last mycotoxin is one of the most potent immunosuppressive agents known, which can be used in organ transplantation to increase the chance of acceptance of the new organ (Gabardi 2011). When animals are exposed to this mycotoxin the immunosuppressive effect can make them vulnerable to infections.

Roquefortine C is known as a neurotoxin and affects the muscles, causing uncoordinated movements. However, to cause any symptoms animals have to be exposed to high concentrations, which are not often found.

5.1 Discussion

All mycotoxins previously discussed can cause adverse health effects in horses, the severity depending on the amount of mycotoxin the horse ingests. Besides these mycotoxins there are new threats, found recently. This may be due to transportation of fungi from another part of the world, or because of changes in the climate, which stimulate mould growth. Another point to be considered are ways to prevent or treat mycotoxin infections. This can be very difficult, due to several factors. We will look into mycotoxin binders, which are meant to bind mycotoxins, so they cannot infect the horses body. The different reasons of why horses are not nearly as thoroughly studied as subjects of mycotoxin infections will also be discussed. Mainly to emphasize the importance of research on mycotoxin infections in horses. Finally another route of exposure will be examined; one which is often forgotten, but has great effects on horses.

5.2 Emerging mycotoxins

A very recent threat to horses in Europe is *Rhytisma. Rhytisma acerinum* is a plant pathogen that affects mostly sycamore and maple trees in summer and autumn. Since the affected areas resemble the effect tar droplets have on leave the disease is commonly called Tar Spots, shown in figure 11. This disease is not considered to be very dangerous to the tree and it therefore usually only controlled with sanitation methods. However, very recently tar spots have been suggested to cause multiple acyl-CoA dehydrogenase deficiency (MADD), which is also known as atypical myopathy, in horses exposed to the infected maple leaves (van der Kolk 2010). Atypical myopathy has many different causes, but in this study all horses had been in pastures near maple trees. Horses have been seen eating fresh leaves, but also leaves that fall of the trees, in autumn. Despite treatment 13 out of 14 horses died. The disease is irreversible and therefore very dangerous, death can follow within 72 hours (Votion 2008). The toxin has not been identified and therefore the mechanism of action is also unknown. More research is needed to determine the impact this disease might have, since no toxicity studies regarding *Rhytisma acerinum* have been performed in mammals yet (to the authors knowledge, van der Kolk 2010).

Figure 11: Maple tree leaf with 'tar spots' caused by *Rhytisma acerinum.*
(Photo by Scimat/Science Photo Library.)

Even without knowing exactly how and what is causing atypical myopathy in horses it is wise to make sure horses are not able to eat maple leaves. Either by removing the entire tree, or by removing all leaves that are within reach of horses. This should prevent the disease from occurring in horses.

5.3 Intervention

It is often difficult to diagnose a horse with a mycotoxin infection due to a few different reasons. Preferentially an analytical measurement of mycotoxins in feed is performed to diagnose a horse. This is often not done due to high costs and the highly variable toxin concentration. Another problem is that a horse may present symptoms several days (or even months in the case of zearalenone) after exposure to mycotoxin infected feed, so the source would no longer be present. The symptoms of a mycotoxin infection can be very similar to the symptoms of other diseases, which also makes diagnosis difficult. The number of mycotoxin infections is likely highly underestimated due to misdiagnosis. It is thought that many cases of colic could be caused by mycotoxins, but again, this is difficult to determine. Also, horses an recover quickly when they are given a different diet, like in an animal clinic, so further investigation is ceased.

When a mycotoxin intoxication is diagnosed in a horse the first thing that needs to be done is to remove the source of the mycotoxins. So all infected straw, hay and other feeds should be taken away from the horse. In some cases this will be sufficient and the horse can make a full

recovery. Other mycotoxins have a lasting effect and the damage may already be irreversible. There are no medicines available that are specifically produced for horses intoxicated with mycotoxins.

5.4 Mycotoxin binders

To prevent mycotoxin intoxication a mycotoxin binder can be added to the feed. There are different types of mycotoxin binders such as clay minerals, organic polymers, activated carbon and aluminosilicates. These binders are used to bind and remove mycotoxins from the gastro intestinal tract (GIT), this way intoxication is prevented by not letting the mycotoxins enter the bloodstream. The mycotoxin binders are added to the horse feed and are supposed to remain in the GIT until they are bound to a mycotoxin, this way they have a lasting effect. Research has been done to determine the efficacy of all these different types of mycotoxin binders, most of which has been done *in vitro* (Whitlow 2006). Most of these studies focus on the binding properties of different substances to *Fusarium*, *Aspergillus* and *Penicillium* toxins and do not include any endophyte mycotoxins.

Some *in vivo* studies have been performed. One yeast cell wall derived mycotoxin binder, glucomannan mycotoxin polymer (GMA) has been used in studies. Yeast cell wall components have many adsorption sites, including hydrogen bonding, ionic and hydrophobic interactions (Huwig 2001). Diaz-Llano *et al* used GMA to show that a group of horses that was fed *Fusarium*-mycotoxin infected diets with the addition of the mycotoxin binder were not affected by the mycotoxin, in contrast to the group that did not have GMA added to their diet (Diaz-Llano 2006). Raymond *et al* showed a loss of appetite in horses that were fed grain contaminated with *Fusarium* mycotoxins. This effect was partially prevented when horses had GMA included in their diet (Raymond 2003). Both of these studies show that mycotoxin adsorbents can have a positive effect on horses. There are many products that claim to adsorb mycotoxins, many of which can be found in Germany. Some brandnames are: Bionit-S, Toxisorb, Bentoniet, Zenifix, Myco Protect, Re-energy, Horsalit, Toxisorb equine, CD vet Toxisorb and Klinofeed, one of these is shown in figure 12.

Minerals can also adsorb mycotoxins. Both clay and zeolite minerals can adsorb mycotoxins that contain a polar group, as is the case with Aflatoxins. They are not good mycotoxin

binders of Fusarium toxins, because they contain less polar groups (Tomasevic-Canovic 2003). It may be possible to alter the minerals so that they do adsorb Fusarium mycotoxins

Activated charcoal (carbon) has been used to bind many different toxins and can be used to reduce mycotoxin infection, but it s not a very effective binder. Because there are more effective and more specific binder activated charcoal is not often used as a mycotoxin binder in feed.

Mycotoxin binders have been investigated for over 20 years and still no product offers complete binding of all mycotoxins. One of the problems is that mycotoxin binders that appear to be effective in *in vitro* tests do not retain their binding qualities when tested *in vivo* (Avantaggiato 2005).

Figure 12: Mycotoxin binder to prevent mycotoxin toxicity. http://www.hiwtc.com/buy/mycotoxin-binder-34112/

So there is a lot of potential gain to be found in mycotoxin binders to help prevent mycotoxins being taken up by the digestive tract (Whitlow 2006). Mycotoxin binders are being added to horse feed already, mainly as a prevention strategy. Because of this, adverse effects of mycotoxin binders should be investigated.

5.5 Inhalation toxicity

Another health hazard for horses is inhalation of fungal spores. As discussed earlier, *Aspergillus* species grow on horse feed post-harvest. Mycotoxins are not the only threat that *Aspergillus* poses to horses. *Aspergillus fumigatus* produces spores that can be inhaled and therefore affect the horses respiratory tract. These *Aspergillus* nanoparticles cause inflammation in the lungs. This route of exposure is difficult to classify because this way horses are not exposed through uptake of mycotoxins by the digestive system, but they are exposed to fungi that are present in the horse feed. Laan *et al* showed that alveolar macrophages increase the expression of cytokine mRNA in response to *in vivo* exposure to *A.*

fumigatus antigen (Laan 2006). These cytokines indicate activation of the immune system. It has been suggested that exposure to *Aspergillus* could lead to COPD (chronic obstructive pulmonary disease) (Larsson 2003). It is thought that many horses that are brought into a horse clinic with airway problems may be suffering from inhalation toxicity from moulds. *Aspergillus* can start to grow inside of tissues and start producing mycotoxins inside the respiratory tract, increasing the exposure to mycotoxins greatly. So when considering *Aspergillus* toxicity not only the mycotoxins found in food should be taken into account, but also the effects caused by the mould itself.

5.6 Conclusion

Concerning mycotoxin toxicity, horses are often not considered in studies due to their high economic and social values.  ddition, the amount of roughages consumed by cattle and other livestock animals is much greater than that consumed by horses.

Another problem is that the focus in studies has been on *Penicilium, Aspergillus* and *Fusarium* mycotoxins. The mycotoxins produced by endophytes, which are a large group of moulds, are often not included. This has different reasons. First of all, endophytes are not visible in the roughage or grain, because they grow inside the plant. So the horse owner does not see the fungus and is not alarmed by the sight of the fungus or a foul odor. Secondly, it is much more difficult to grow endophytes in a laboratory, so research on this type of fungus is not easily done.

Furthermore, the amount of mycotoxins has increased greatly during the past years. This may be due to changes in the climate. Since most moulds grow well under moist conditions, more precipitation and an increase in temperature could increase the amount of mould growth. Also new moulds are being found affecting horses in Europe, like *Rhytisma*. Since there are no medicines available and prevention methods are not always effective this increase in moulds and their mycotoxins can be a serious threat to our horse population.

Considering all these points it can be concluded that it is of importance to investigate mycotoxins in horse feed more thoroughly, including ways to prevent and treat intoxications.

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