Symptoms, Diagnosis, and Pathophysiology of Mycotoxin Exposure

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INTRODUCTION

Mycotoxins are secondary metabolites produced by a variety of pathogenic fungi. Common mycotoxins of human and veterinary interest are produced by species in the genera *Aspergillus, Claviceps, Fusarium,* and *Penicillium*. There are over 100 known species of fungi that produce mycotoxins. Some species of fungi produce single mycotoxins, while others produce multiple toxins. It is important to mention that the contamination of food/feed with fungi and their mycotoxins can occur at any stage of the food production chain, i.e., from the time of planting through harvesting, transportation, storage, and until the food/feed is consumed.

Animal poisonings by mycotoxins, commonly referred to as "mycotoxicosis," have been recorded for centuries. The oldest known mycotoxicosis involving mass poisoning in both man and animals is associated with ergot and ergot-related alkaloids produced from *Claviceps purpurea*. From time to time, several large outbreaks of mycotoxins have occurred in many countries. Alimentary toxic aleukia (ATA) reported in eastern Siberia from 1942 to 1947 was caused by a species of *Fusarium* growing on grain that had overwintered on the ground. In the 1930s, horses were poisoned in Russia by eating straw infected with *Stachybotrys chartarum* known to produce the mycotoxin, satratoxin H. Over 5,000 horses died in 1934 and 1935 from equine leukoencephalomalacia (ELEM) in central Illinois and some other parts of the Midwest after consuming corn infected with *Fusarium verticillioides* (formerly *Fusarium moniliforme*) and *Fusarium proliferatum*. In 1989, these fungi were shown to produce mycotoxins identified and confirmed as fumonisins. During 1989 to 1992 in the United States, a significant number of horses died from consuming corn contaminated with fumonisins. Horses were also intoxicated in 1966 in the United States by consuming barley contaminated with *Fusarium graminearum* (barley scab). Yearly losses in livestock, particularly in horses, due to mycotoxicosis are enormous throughout the world. Unlike ruminants, horses are more sensitive to mycotoxins because of their limited detoxification capability in the gastrointestinal tract.

There are hundreds of fungal metabolites that are potentially toxic to animals. This paper describes the toxicity of some commonly occurring mycotoxins (such as aflatoxins, fumonisins, slaframine, trichothecenes, and zearalenone) in equine species.

Aflatoxins

Aflatoxins were discovered as a result of massive losses of turkeys in Great Britain in 1960. Over 100,000 turkeys died in the outbreak, which was called "turkey X disease." The cause of death was identified as aflatoxin, found in moldy groundnut meal imported from Brazil. Since then, sporadic incidences of aflatoxicosis have occurred in many species around the world. Almost all livestock animals are sensitive to the toxicity of aflatoxins, but a great variability exists in that sensitivity.

There are four major aflatoxins of toxicological importance: aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2). These four aflatoxins were named according to their fluorescent properties under long wavelength ultraviolet light on thin-layer chromatography (TLC) plates. Aflatoxins B_1 and B_2 fluoresce blue, whereas aflatoxins G_1 and G_2 fluoresce green. The subscript numbers 1 and 2 indicate major and minor compounds, respectively. AFB_1 and AFB_2

are hydroxylated and excreted in the milk as AFM_1 and AFM_2, which are of a lower toxicity than the parental aflatoxins. There are more than fourteen metabolites of the four major aflatoxins that have been chemically characterized. Certain environmental factors and insects usually damage corn, small grains, peanuts, cottonseed, etc., and thereby promote the growth of fungi and the production of aflatoxins.

Aflatoxins are mainly produced by *Aspergillus flavus, A. parasiticus*, and *A. nomius*. Other fungi known to produce aflatoxins are *A. bombycis, A. fumigatus, A. ochraceus*, and *A. pseudotamarii*. Details of aflatoxigenic species of *Aspergillus* and their worldwide distribution are described in recent publications (Coppock et al., 2012; Varga et al., 2009). Among all these fungi, *A. flavus* has been most extensively studied for the production of aflatoxins. The production of aflatoxins is associated with spore production by species of *Aspergillus* (Calvo et al., 2002). Strains of *A. flavus* can vary in aflatoxin capability from nontoxic to highly toxigenic and are more likely to produce more AFB_1 than aflatoxin AFG_1. *A. flavus* and other species can also produce cyclopiazonic acid (CPA). Strains of *A. parasiticus* generally have less variation in toxigenicity and generally produce AFB_1 and varying amounts of AFB_2, AFG_1, and AFG_2. The aflatoxin profile produced by *A. nomius* is considered to be similar to *A. parasiticus*, and like *A. parasiticus, A. nomius* does not produce CPA. Recent studies have shown that *A. nomius* is more important as a producer of aflatoxins than previously suspected (Johnson et al., 2008). Aflatoxigenic strains of *Aspergillus* can also produce sterigmatocystin.

Toxicity and Clinical Signs

The primary aflatoxins of toxicological concern in feedstuffs are AFB_1, AFB2, AFG_1, and AFG2. On a functional basis, analyses for aflatoxins generally are the sum of the concentrations of these four toxins (Coppock et al., 2012). Among all aflatoxins, AFB_1 occurs with the greatest frequency in feedstuffs and is found to be the most toxic in all laboratory, companion, and livestock animals. In general, the order of toxicity is AFB_1> AFG_1> AFB_2> AFG_2. The LD50 of aflatoxin B_1 in ducklings, rabbits, dogs, and guinea pigs is about 1 mg/kg. This value is about 10 mg/kg in monkeys, cattle, pigs, rats and hamsters. Mice and sheep are less sensitive to the toxicity of aflatoxin B_1, as the LD50 values are 63 and 500 mg/kg, respectively.

Toxicity due to aflatoxins, under natural conditions, is usually subacute or chronic, depending on the level of exposure. Occasionally, acute cases are also seen. In general, affected animals show reduced growth rate, weight loss, immune suppression, icterus, hemorrhagic enteritis, reduced performance, and ultimately death.

The major target for the toxicity of aflatoxins is the liver. AFB_1 is known to cause hepatocellular carcinomas (HCC) in many animal species and in humans. The occurrence of HCC can vary with age of exposure. For example, brief exposures to large doses of AFB_1 during the neonatal period result in a high incidence of HCC in adulthood, whereas adult mice exposed to the same doses fail to develop HCC at any age. Aflatoxins produce necrosis of liver cells, damage to mitochondria, and proliferation of bile ducts. The clinical pathology of aflatoxicosis has been studied in several animal species, including horses. Weanling ponies were administered AFB_1 at 0.0 (control), 0.5, 1.0, and 2.0 mg/kg (Boatell et al., 1983). Serum activity of gamma-glutamyl transpeptidase (GGT) was increased in all ponies receiving AFB_1. The GGT activity remained increased until day 3 and then decreased. Serum activity of alanine aminotransferase (ALT) remained unchanged. Ponies given 4, 5, 6, and 7.4 mg AFB_1/kg body weight had an increase in serum ALT, and the activity of ALT remained increased until the ponies died at 33 to 46 hours after dosing.

Animals poisoned with aflatoxins may show hemorrhage into the gastrointestinal tract and body

cavities and on body organs due to a decreased production of clotting factors by the liver (Boatell et al., 1983; Greene and Oehme, 1976). At necropsy, common findings include firm and pale liver, clear yellow ascites, and pleural fluid accumulation. Histopathological lesions are commonly reported in liver and kidney. Angsubhakorn et al. (1981) reported hemosiderin deposition in tubule cells, cardiac myofiber degeneration, and edema of the brain in horses. Hepatic encephalopathy could occur as a result of liver damage.

Reproductive and Developmental Effects

Many reports describe deleterious effects of aflatoxins on the reproductive and developmental systems such as sexual maturation, growth and maturation of the follicles, levels of hormones, gestation, and growth of the fetus (Kourousekos and Lymberopoulos, 2007; Turner et al., 2007; Gupta, 2011).

In many in vivo and in vitro studies, aflatoxins have been investigated for male reproductive toxicity, and the principle target organ is testes, and of course, various aspects of spermatogenesis. Aflatoxins are known to cause testicular degeneration, sloughing of germ cells, and concomitant reduction in the rate and efficiency of sperm production. Recently, Shuaib et al. (2010) demonstrated that AFB_1 caused regression of the testes, impairment of spermatogenesis, and premature loss of germ cells. An intraperitoneal injection of 50 µg of AFB_1/kg /day (estimated to be equivalent to ~330 ppm in diet) was given to male mice at various intervals (Agnes and Akbarsha, 2003). At day 35, fertility testing showed a decrease in litter size, and tissue examination showed a decrease in spermatozoa numbers present in the caudal epididymis. When the numbers of spermatozoa decreased, forward mobility of spermatozoa was decreased and abnormal spermatozoa were observed. It is important to mention that toxicants that target spermatogenesis and spermiogenesis may not be apparent for 3 to 6 weeks, depending on the species, due to the time required for spermiogenesis.

AFB_1 has also been shown to impair the reproductive performance of female animals (Ibeh and Saxena, 1997a,b). Female rats (Druckery Strain) receiving AFB_1 (7.5 mg/kg, po for 21 days) showed significant reductions in the number of oocytes and large follicles in a dose-related manner. Blood hormone levels and sex organ weights were significantly altered. There were reductions in ovarian and uterine sizes, increases in fetal resorption, implantation loss, and intra-uterine death in aflatoxin-exposed female rats.

It is important to mention that AFB_1 in animals and humans crosses the placental barrier and thereby reaches the fetus (Partanen et al., 2010; Gupta, 2011). High AFB_1 concentrations in the umbilical cord have been associated with low birth weight, kernicterus, and in some cases also with death of the fetus (Abdulrazzaq et al., 2004). Chronic exposure to AFB_1 may cause endocrine disruption in the fetoplacental unit, as it has been shown to affect the expression of the aromatase enzyme (Storvik et al., 2011). These authors demonstrated that AFB_1 had effects on genes important to endocrine regulation in placental cells. The developmental toxicity of AFB_1 has been studied in various laboratory animal models, and this mycotoxin has been found to be embryotoxic and/or teratogenic in rats, mice, hamsters, chick embryos, tadpoles, and Japanese medaka eggs. Evidence suggests that aflatoxins are teratogenic to most animal species (WHO, 1990; Wangikar et al., 2005; Gupta, 2011).

Treatment

There is no specific antidote for toxicity of aflatoxins. Timely administration of L-methionine (200 mg/kg) and sodium thiosulfate (50 mg/kg), at eight-hour intervals, is proven to be of therapeutic

value. Supplementation with increased levels of protein, vitamins, and antioxidants can also be rewarding.

Immediate removal of contaminated feed is the single most important step in avoiding any further loss to animal productivity and/or death. The FDA's goal for aflatoxins has been to minimize contamination by implementing regulations that focus special attention on the management of the problem. Currently, less than 20 ppb aflatoxin B_1 in feed is considered to be safe.

Biological exposure of aflatoxins can be minimized by chemoprotection and/or enterosorption. Chemoprevention against aflatoxins has been demonstrated with the use of a number of compounds (such as esterified glucomanoses and other yeast extracts) that either increase detoxification of aflatoxins or prevent the production of the aflatoxin epoxide, thereby reducing or blocking AFB_1- induced hepatocarcinogenesis. Compounds such as oltipraz and chlorophyll are available to decrease the biologically effective dose. Enterosorptive food additives are recommended because they bind aflatoxins and render them biologically unavailable to humans and animals (Williams et al., 2004). Dietary supplementation with a feed anti-caking agent or adsorbents, such as 0.5% hydrated sodium calcium aluminosilicate (HSCAS or NovaSil), has been demonstrated to minimize the aflatoxicosis problem in a number of species. Selective calcium montmorillonites have proven to be the most selective and effective of these enterosorbents. Following absorption, some zeolites can be effective reactive oxygen species (ROS) scavengers (Pellegrino et al., 2011). The efficiency of mycotoxin binders differs considerably depending mainly on the chemical structure of both the adsorbent and the toxin (Huwig et al., 2001). It is important to mention that by no means should these binders be considered mycotoxin eliminators. One disadvantage with these binders is that there can be some interference in the absorption of essential nutrients. This factor should definitely be taken into consideration, especially during pregnancy and the fetal developmental period.

Fumonisins

The discovery of the fumonisins (FB_1, FB_2, and others) was a major breakthrough in the identification of mycotoxin(s) involved in moldy corn poisoning in equine species associated with corn or feeds contaminated with *Fusarium verticillioides* (formerly *F. moniliforme Sheldon*) or *F. proliferatum* (Gelderblom et al., 1988; Marasas et al., 1988; Marasas, 2001). Some fumonisins and related mycotoxins in minor amounts are also produced by other *Fusarium* spp., such as *F. subglutinans*, *F. anthophilum*, and *Aspergillus niger* (Månsson et al., 2010). More than 30 fumonisins have been identified, and still new stereoisomers such as epi-FB_3 and epi-FB_4 and novel congeners, such as fumonisin B6, continue to be discovered and structural confirmation of more is likely (Bartók et al., 2010; Månsson et al., 2010). FB_1 is the predominant isomer and usually accounts for 60% or more of the fumonisins in corn. FB_2 and FB_3 occur in small amounts with FB_2 being more prevalent.

Problems related to fumonisins are common throughout the world. Fumonisins are known to cause equine leukoencephalomalacia (ELEM), commonly referred to as "moldy corn poisoning," in equine species. Fumonisins have been implicated in the production of pulmonary edema or "hydrothorax" in pigs, esophageal cancer in humans, liver cancer in rats and mice, and immunosuppression in chickens.

Toxicity and Clinical Signs

Fumonisins have been known to adversely affect the brain, lungs, liver, esophagus, kidneys, pancreas, testes, thymus, gastrointestinal tract, and blood cells. Depending upon the species, some tissues are more affected than others.

Horses are the most sensitive species for fumonisins (Voss et al., 2007). Classically, horses ingesting contaminated feed develop a syndrome referred to as equine leukoencephalomalacia (ELEM). As little as 5 ppm fumonisin B_1 in corn can cause ELEM in 7 to 35 days. Symptoms reflect neurological injury and include ataxia, head pressing, paralysis of the lips and tongue, and convulsions. The disease progresses rapidly, and the mortality rate is near 100%. At necropsy, one or more foci of hemorrhagic liquefactive necrosis are characteristically present in the white matter of the brain, predominantly in the cerebrum.

A hepatotoxic syndrome also occurs in horses, characterized by icterus, elevated concentrations of serum bilirubin and liver enzymes, and swelling of the face and head. Necropsy usually reveals a small, firm liver and, upon microscopic examination, centrilobular necrosis and periportal fibrosis. Neurotoxic signs may also develop before death (Voss et al., 2011). In general, high doses of fumonisins induce fatal hepatotoxicity with mild brain lesions, whereas low doses cause mild hepatotoxicity and severe brain lesions characteristic of ELEM. Fumonisin-induced liver damage also occurs in other species, including pigs, cattle, rabbits, and primates.

In horses, FB_1 also causes cardiotoxicity (Smith et al., 2002). Decreased heart rates and cardiac contractility and increased systemic vascular resistance were found in horses exposed to 0.2 mg/kg FB_1 administered intravenously. Repeated daily doses of 0.01 mg/kg FB_1 over 28 days caused no cardiovascular impairment but did affect sphingolipid profiles of cardiac tissue (Foreman et al., 2004).

Accumulation of sphinganine, sphingosine, and their 1-phosphates in tissues, blood, or urine are useful biomarkers of fumonisin exposure and a close correlation between increased sphingoid base concentrations, especially those of sphinganine, and toxicity have been shown in experiments in many species, including horses.

Fumonisins are also known to adversely affect the immune system. Several studies have demonstrated that fumonisin B_1 affects innate immunity, as well as humoral and cellular responses of acquired immunity (Bondy and Pestka, 2000; Bhandari and Sharma, 2002; Theumer et al., 2002; Mishra and Sopori, 2012). Exposure to FB_1 was reported to cause localized activation of the cytokine network, suggesting that the toxin induced changes in innate immune responses may be important in its immunotoxicity (Bhandari and Sharma, 2002).

Recently, Voss et al. (2011) described in detail the reproductive and developmental effects of fumonisins in various animal species. Abdel-Wahhab et al. (2004) reported that FB_1 proved to be teratogenic and induced severe fetal growth retardation and skeletal malformation. In many studies, fumonisin exposure has been linked to neural tube defects (Missmer et al., 2006; Voss et al., 2007).

Diagnosis of fumonisin toxicity can be made based on: (1) detection of fumonisins (FB_1 and FB_2) in feed or animal tissue/fluid, and (2) increase in the sphinganine to sphingosine ratio in serum and tissue.

Treatment

There is no specific treatment for fumonisin toxicity in horses. Immediate removal of contaminated corn or feed is the single most important step in preventing other animals from developing signs.

Slaframine

Slaframine (6-aminooctahydroindolizin-1-yl acetate), commonly referred to as the "slobber factor," is produced by the fungus *Rhizoctonia leguminicola*. The fungus is ubiquitous in soil and parasitizes red clover (*Trifolium pratense*), white clover (*Trifolium repens*), alsike clover (*Trifolium hybridum*), and many other legumes, and causes a pasture disease called "black patch." Fungal

infestation is usually associated with periods of wet and cool weather. Spring and fall are ideal for the growth of *R. leguminicola*.

Slaframine is known to produce excessive salivation in many animal species, including sheep, swine, and guinea pigs, but more importantly in cattle and horses (O'Dell et al., 1959; Croom et al., 1995). A serious outbreak of slobbers occurred in a herd of horses in the fall of 1979 near High Point, North Carolina. This was caused by a shipment of high-quality second-cutting red clover/orchard grass hay from a supplier in West Virginia (Hagler and Behlow, 1981). Since then, problems with slaframine have been noted in many parts of the United States, particularly in the Midwest. In Kentucky and surrounding states, problems associated with slaframine are quite common in horses. In 2010, problems related to slaframine toxin were observed in Central Kentucky because of the wet spring weather and abundant clover growth (Gaskill, 2011). It is important to note that slaframine is not destroyed during drying of plants or during the process of making silage or haylage.

Toxicity and Clinical Signs

The clinical signs of slaframine toxicity are similar in all species. In experimental studies, animals exposed to a single dose of slaframine showed salivation for 6 to 10 hours. Slaframine exposure at above 10 ppm concentration can be associated with slobbering. Naturally occurring cases of slaframine poisoning are primarily reported in horses and ruminants. Slobber syndrome usually occurs soon after consumption of hay or forage contaminated with slaframine. In addition to excessive salivation, horses usually show signs of lacrimation, colic, and diarrhea. One case report described abortion in a mare (Smith and Henderson, 1991). Excessive salivation may persist for several days, even after withdrawal of the contaminated source. Clinical signs in cattle, in addition to salivation, are lacrimation, bloating, frequent urination, and watery diarrhea. Other clinical signs may include anorexia as well as irregularities in the gastrointestinal, cardiac, and respiratory systems. Weight loss, decreased milk production, uterine hemorrhage, and abortions have also been associated with slaframine toxicosis. Fatalities with slaframine toxicosis are not common. However, death may occur due to suffocation from pulmonary edema and/or emphysema.

Diagnosis of slaframine toxicity is based on clinical signs (particularly profuse salivation) in animals consuming red clover or some other legume forage, identification of *R. leguminicola*, and detection of slaframine/swainsonine in forage, plasma, or milk (Imerman and Stahr, 1998).

Treatment

Therapy with atropine sulfate is quite effective in combating the clinical signs associated with parasympathomimetic action. However, it is unlikely that clinical signs will be completely resolved (Smith, 2012). In laboratory animals, antihistamines such as promethazine hydrochloride have also been found effective. Recovery of animals is usually rapid, as the prompt removal of the contaminated source generally alleviates all signs of intoxication within 2 to 5 days.

Trichothecenes

Trichothecene mycotoxins occur worldwide and their impact on animal and human health is of global concern. Trichothecenes were named after the fungus *Trichothecium roseum*, from which the first trichothecene was isolated in 1948. Today, approximately 190 trichothecenes have been isolated and identified from *Fusarium, Myrothecium, Verrucaria*, and other genera. The major mycotoxins of the trichothecenes group are deoxynivalenol (DON or vomitoxin), diacetoxyscirpenol (DAS), and T-2 toxin. These three closely related trichothecene mycotoxins are most commonly found in

agricultural commodities infected with *Fusarium* species. These fungal metabolites are a group of sesquiterpenoids characterized by a tetracyclic 12,13-epoxytrichothec-9-ene skeleton and a variable number of acetoxy or hydroxyl group substitutions. All trichothecenes share a tricyclic nucleus named trichothecene and usually contain an epoxide at C-12 and C-13, which is considered essential for toxicity. Trichothecenes can be broadly divided into two groups, macrocyclic and non-macrocyclic, based on the presence of a macrocyclic ring linking C-4 and C-15 with diesters (roridin series) and triesters (verrucarin series) (Mostrom and raisbeck, 2012).

Vomitoxin is produced by the fungus *Fusarium roseum*, whereas DAS and T-2 toxin are produced by several fungi, such as *F. tricinctum, F. solani, F. roseum*, and *F. nivale*. Production of these mycotoxins is usually triggered by a variety of environmental conditions, such as cool and wet weather. Trichothecenes have been known to contaminate a variety of field crops, including wheat, barley, rice, oats, corn, sorghum, and sunflower. During the past four decades, it has been noted that among trichothecenes, vomitoxin occurs with the greatest frequency. Humans and almost all livestock animals are sensitive to trichothecene toxicity. Dogs and pigs are more sensitive to vomitoxin than other species. Occurrence of toxicosis in domestic animals involving T-2 toxin or DAS is rare. Although DON occurs as a feed contaminant with greater frequency, T-2 toxin has been studied in greater detail because of its relevance to chemical warfare. It is important to note that trichothecenes are stable for long periods, and as a result, their concentrations generally magnify during feed processing and storage.

Toxicity and Clinical Signs

Trichothecenes are toxic to all animal species that have been tested. Toxicity of trichothecenes appears to vary as verrucarins and roridin E are the most acutely toxic trichothecenes, followed by DAS and T-2 toxin, and nivalenol and crotocin are least toxic (Ueno, 1983; Li et al., 2011). Furthermore, neonatal animals are more susceptible than adults to the toxicity of trichothecenes. Their toxicity also varies from species to species. Based on toxicity, the species susceptibility to DON are ranked as pig> rodent> dog> cat> poultry> ruminants (least sensitive) (Prelusky et al., 1994). No such information is available for equine species.

The toxic effects may depend upon the route of exposure, concentrations of trichothecenes in the diet, and the duration of exposure. Clinical signs can range from subtle effects such as feed refusal or reduced feed consumption, decreased weight gain, skin irritation, and increased susceptibility to diseases to the more severe effects such as bloody diarrhea, complete anorexia, emaciation, and finally death. It is demonstrated that as little as 1 ppm vomitoxin in feed can cause feed refusal in pigs and dogs. Refusal concentrations for DAS and T-2 toxin in pigs are 10 ppm and 16 ppm, respectively. Trichothecenes are known to cause dermal necrosis, gastrointestinal effects, hemorrhage, coagulopathy, and immunosuppression. T-2 toxin is associated with a large range of toxic effects, such as weight loss, decreases in blood cell and leukocyte counts, reduction in plasma glucose, pathological changes in the liver and stomach, and alimentary toxic aleukia (ATA). Animal feeding experiments also demonstrated that trichothecenes are teratogenic but provided no evidence that they are carcinogenic (Anonymous, 1983).

Field cases of lethality in horses from trichothecene mycotoxicoses (*Stachybotrys*) in the Soviet Union have been recorded since the 1930s (Forgacs, 1972; Dankø, 1975; Hintikka, 1978). Stachybotryotoxicosis typically occurred during indoor feeding of horses with *S. alternans*-contaminated straw or hay. Horses are very sensitive to *Stachybotrys* toxin, and 1 mg of toxin can be lethal. In the peracute form of stachybotryotoxicosis, which is associated with high concentrations of *Stachy-*

botrys toxin in the feed, horses show signs of nervous system irritation or depression, cardiac arrhythmias, pulmonary edema, hemorrhages of serosal and mucosal membranes and muscular tissue, muscle necrosis, and oral ulcers (Hintikka, 1978). Hemorrhage occurs on serous and mucous membranes and in the spleen, liver, lungs, brain, spinal cord, lymph nodes, and most notably in muscle tissue. In the early 1970s, horses died in northern Japan from consuming moldy bean hulls, which were commonly used as fodder and bedding especially in the winter and spring (Ishii et al., 1971). The affected horses showed clinical signs of CNS disturbances including convulsions and cyclic movements, depressed respiration, icterus, and bradycardia. Poisoning in these horses was linked to several trichothecenes (T-2 toxin, DAS, and neosolaniol).

In an experimental study conducted by Johnson et al. (1997), five horses (about 444 kg) were fed DON-contaminated barley (about 36 to 44 ppm DON), and they consumed approximately 1.27 kg barley/horse/day (about 0.099 to 0.124 mg DON/kg/day) for 40 days. No signs of feed refusal were observed in the horses, and no remarkable changes were detected in any hematological or serum biochemical parameters. Both serum IgG and IgA decreased in a linear manner through the trial. The authors suggested that gastric microflora detoxified DON prior to absorption. In another trial performed by Raymond et al. (2005), mares were fed one of three treatments, a control diet, a mycotoxin diet (11.2 mg DON/kg and 0.7 mg 15-acetyldeoxynivalenol/kg diet), and a mycotoxin diet (14.15 mg DON/kg feed and 0.7 mg 15-acetyldeoxynivalenol/kg diet) with 0.2% glucomannan polymer (an adsorbent) for 21 days. Feed intake and body weight gains were depressed in horses fed the mycotoxin-contaminated diets as compared with control mares. No effect of diet was seen on hematology or serum chemistries, which included gamma-glutamyltransferase activity, nor were any differences noted in athletic ability. Feeding glucomannan polymer did not prevent a depression in feed consumption.

Treatment

The most effective control strategy for trichothecene toxins is prevention of fungal infection and toxin production in the field and in storage. Proper agricultural practices such as avoiding late harvests, removing overwintered stubble from fields, and avoiding a corn-wheat rotation that favors Fusarium growth in residue can reduce trichothecene contamination of grains. Storage of grains at less than 13 to 14% moisture and hay and straw at less than 20% moisture are important in preventing trichothecene production.

There is no specific treatment for trichothecene mycotoxicoses. A number of binders such as clay and zeolitic products have been suggested for use with trichothecene-contaminated feed to prevent absorption by animals. However, the USFDA has not approved any chemical for use as a trichothecene mycotoxin binder. Toxic effects can be alleviated by replacing contaminated feed with clean feed. Studies show that washing of corn for 48 hours, with a change of water every two hours, is very effective. Clinical signs of feed refusal usually disappear within a week after removal of the contaminated feed, and animals return to production within14 days.

For further details on trichothecenes, readers are referred to a recent publication by Mostrom and Raisbeck (2012).

Zearalenone

Zearalenone, also referred to as F-2 toxin, is chemically described as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-ß-resorcylic acid lactone. At least seven derivatives of zearalenone naturally occur in corn (Mostrom, 2012). Zearalenone and its derivatives are also commonly found in barley, oats,

wheat, corn silage, rice, sorghum, and occasionally in forages. The occurrence of zearalenone in grain and feed is worldwide, especially in temperate climates. Zearalenone is primarily produced by *Fusarium graminearum*, but it can also be produced by other *Fusarium* fungi, including *F. culmorum, F. verticillioides* (formerly *F. moniliforme*), *F. sporotrichioides, F. semitectum, F. equiseti*, and *F. oxysporum*. Moisture content and the presence of oxygen are critical factors for zearalenone production. If the fungus is stressed by a cool temperature of 8 to 15°C for several weeks, zearalenone can be produced. This mycotoxin can be produced fairly quickly in the field during wet weather in the late summer or early fall weather following hail damage to corn. Very high concentrations of zearalenone can also be found in grain stored improperly at high moisture. Corn stored in a crib and exposed to winter is particularly prone to fungal invasion and production of zearalenone. It needs to be emphasized that in addition to the co-occurrence of other estrogenic metabolites, such as α- and ß-zearalenol, zearalenone is commonly detected in grain with deoxynivalenol. Zearalenone and its metabolites are heat stable.

Following oral ingestion, zearalenone is rapidly absorbed from the gastrointestinal tract. In monogastric animals, bioavailability of zearalenone can be estimated over 80%.

Toxicity and Clinical Signs

In general, zearalenone has a low acute toxicity in most animal species. A wide variability also exists in species sensitivity to zearalenone toxicity. Prepubertal swine are the most sensitive and poultry appears to be the least sensitive species. Also, females are more sensitive than males and the young are more sensitive than the old. Gimeno and Quintanilla (1983) reported estrogenic signs of edematous vulvas, prolapsed vaginas, oversized uteri, and internal hemorrhage in mares and severe flaccidity of genitals in two male horses fed corn screenings for 30 days in a field exposure. All sick animals collapsed with respiratory paralysis and sudden blindness, and died quickly. Feed analysis revealed zearalenone at 2 to 3 mg/kg. Unfortunately, the feed was not analyzed for fumonisin, which is known to cause blindness. In another study conducted on six cycling trotter mares, Juhász et al. (2001) determined that daily oral administration of 7 mg purified zearalenone starting ten days after ovulation and continued until the subsequent ovulation had no adverse effect on reproduction. The dose of purified zearalenone represented a natural contamination of feed of about 1 mg zearalenone/kg feed and ranged between 0.013 and 0.010 mg zearalenone /kg body wt/day for approximately 8 to 10 days. In this study, zearalenone produced no effect on the length of the interovulatory intervals, luteal or follicular phases of the ovary, and did not significantly affect uterine edema. It was recognized that the exposure period to zearalenone was short.

Treatment

There is no specific antidote for zearalenone toxicity. Symptomatic treatment is advisable. Replacement of contaminated feed with clean feed allows recovery from estrogenic signs within 1 to 2 weeks. Within 3 to 7 weeks following removal of the contaminated feed, animals will return to normal reproductive status. No zearalenone binder has been proven to be efficacious and there are none currently approved by the US Food and Drug Administration (Mostrom, 2012).

CONCLUDING REMARKS

Mycotoxicoses usually occur from consuming mycotoxin contaminated feed, affecting equine health worldwide. The economic loss is hundreds of millions of dollars each year. This paper has described the toxicity of some commonly encountered mycotoxins such as aflatoxins, fumonisins,

slaframine, trichothecenes, and zearalenone in equine species. Fumonisins and aflatoxins are the two most frequently encountered mycotoxins in feed causing mycotoxicoses in equine species. Evidently, each mycotoxin adversely affects equine health by a single or multiple mechanism of action, involving selected target organ(s). There is no specific antidotal treatment for any mycotoxin. A number of binders have been tested and they offer some beneficial effects. However, these binders can also cause a nutritional deficiency of some elements. Therefore, replacement of contaminated feed with clean feed appears to be the best solution. Future research is needed to understand the detailed mechanism of action of these mycotoxins so that effective antidotes can be developed.

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