Mycotoxins in Forages and Their Impact on Animal Health

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Abstract

Moldy and damaged forages occur commonly and are widely fed to ruminants. Major mycotoxins associated with forages include the endophyticfungi responsible for fescue toxicosis, the tremorgens, slaframine associated with red clover, stachybotryotoxin in straw, sporodesmin, and ergot alkaloids from the grasses. Effects from forage associated mycotoxins are seldom acutely fatal, but cause effects including reduced gain, lowered milk production, peripheral gangrene, ataxia and tremors, excessive salivation, gastroenteritis, photosensitization and dermal necrosis. Clinical effects can mimic other common disease conditions and diagnosis depends on thorough review of history and circumstances as well as clinical and laboratory evaluation. Currently, analytical tests are used to confirm presence of the toxins, but are not routinely available on a routine basis. Common feed grain mycotoxins (aflatoxin, zearalenone, trichothecenes) are occasionally reported from forages, but generally are not a clinical or production problem associated with forages. Most of the forages related mycotoxins are associated with well defined climatic, weather and cultural conditions; thus often can be avoided by appropriate cultural and management practices. Prevention is the key to reducing losses since specific effective antidotes are not available.

General Considerations

The spoilage of feeds and forages by fungi depends on both the environment and the substrate on which the fungus grows. Additionally, the invasion of forages by certain fungi growing within the plant structure itself may establish a symbiotic relationship beneficial to both organisms. These fungi, known as endophytes, occur in fescue and rye grass where they induce formation of toxins detrimental to livestock. In some of the well known forage mycotoxicoses, fungal infection and mycotoxin production are not apparent as a pathogenic effect on the plant. Unfortunately, compared to mycotoxins of feed grains, relatively little is known about the toxic animal effects of forage fungi. This discussion will cover major effects of known mycotoxins in forages, with some comparisons to the more familiar grain-related mycotoxins, and will suggest approaches to prevention and management of these problems as part of herd health management.

Mycotoxins are toxic secondary metabolites produced by fungal (mold) growth. A secondary metabolite is not essential to normal structure or function of the mold. Secondary metabolites are produced sporadically in response to stress. Not all secondary metabolites are toxic. Mycotoxin occurrence is often associated with geographic areas that support environmental conditions for mold growth and concurrent stress of the fungus. Table 1 indicates regions of North America most likely to support growth of fungi that produce feed and forage mycotoxins.

Fungi of feed grains that grow on susceptible crops prior to harvest are known as field fungi. Among these, *Fusarium* is the most common producer of several important mycotoxins. *Fusarium* generally requires relative humidity above 90% and rarely grows after harvest because it is usually below critical moisture needs. Growth in storage generally requires moisture concentrations of 22-25%.

Storage fungi (e.g. *Aspergillus* and *Penicillium*) typically do not invade intact grain prior to harvest unless stress conditions such as drought or insect damage predispose them to infection in the field. Fungal growth requires a proper substrate (carbohydrate), moisture, oxygen and adequate temperature for growth. Formation of some toxins, such as aflatoxin may require prolonged periods where the minimum daily temperature does not fall below a critical level. This critical minimum temperature combined with stress conditions related to insects and drought or heat stress is why aflatoxin is primarily a problem in tropical or subtropical regions.

Biologic Effects of Mycotoxins

The biologic effects of mycotoxins result from their interference with basic biochemical or hormonal processes in the cell. Some mycotoxins affect metabolic and anabolic processes by inhibiting mitochondrial function and altering enzymes that promote carbohydrate metabolism. Others interfere with lipid metabolism, reducing fatty acid and cholesterol biosynthesis. A third major biochemical effect of mycotoxins is impaired nucleic acid function resulting in reduced or altered nucleic acid orprotein biosynthesis. This effect on protein synthesis reduces the synthesis of a multitude of enzymes, and the effects may not be manifest until existing enzymes are

Table 1. Quick Guide to Mycotoxins That Affect Livestock

Toxin	Geographic Reference	Substrates	Fungus	Disease Manifestations
Aflatoxin B_1, B_2, G_1, & G_2	South-East and South Central USA; From drought and insect stress	Cottonseed Corn Peanuts Sorghum	Aspergillus flavus Aspergillus parasiticus	Hepatotoxicosis, hepatic carcinogenesis, cholangio-hepatitis, hemorrhage coagulopathy. Slow growth, poor feed conversion, reduced milk production. Milk residues are regulated.
Fumonisins	Corn Belt and all USA where corn is grown	Corn	Fusarium moniliforme	Cattle tolerate up to 50 ppm with no adverse effects. Above 100 ppm may cause liver damage and immune suppresssion
Ochratoxin A	North Central USA and Canada	Corn, Wheat Peanuts	Aspergillus ochraceus Penicillium viridicatum	Nephrotoxicosis with polydipsia and polyuria. Weight loss and anorexia.
Citrinin	North Central USA and Canada	Wheat, Rye Oats, Barley	Penicillium citrinum	Similar to ochratoxin
Trichothecenes (e.g. T-2 toxin, diacetoxyscirpenol, deoxynivalenol vomitoxin, DON)	North Central USA, Canada, Appalachia and Mid-South	Corn Barley Wheat Sorghum	Fusarium sporotrichioides	Feed refusal, diarrhea, weight loss, necrotic dermatosis. Lympholysis and thymolysis. Feed refusal is most common sign in natural exposure. Some data show reduced milk production.
Zearalenone (F-2) and Zearalenol	North Central and Central USA, Canada	Corn Sorghum Wheat	Fusarium roseum	Estrogenic vulvovaginitis in gilts, anestrus infertility, and early embryonic death in sows. Mammary enlargement and infertility in cows.
Tremorgenic mycotoxins (Penitrems, fumitremorgen)	Pacific Coast & North Central USA	Corn, Peanuts Pecans Walnuts	Penicillum cyclopium Aspergillus spp.	Tremors, convulsions, low mortality, Recovery when source removed
Tunicamycin and Lolitrem B	Pacific Northwest and California	Perennial ryegrass	Acremonium lolii	Stiffness, incoordination, tremors, hypermetria, opisthotonus, seizures, recumbency, high morbidity and low mortality.
Penitrems	South Central and South-East USA	Dallis grass	Paspalum dilatatum	Tremors, ataxia, excitement, seizures, recumbency. Rapid recovery when source is removed.
Ergotamine and related alkaloids	North & Central USA (Small Grain Area)	Rye Cereal grains	Claviceps purpurea	Gangrene of the extremities; convulsions, ataxia, and tremors, agalactia due to reduced prolactin.
Ergovaline	South-East USA	Fescue forage	Acremonium coenophialum	Heat intolerance, infertility, reduced prolactin
Slaframine	Midwest & Eastern to North-East USA	Red Clover	Rhizocotonia leguminicola	"Slobbers syndrome", salivation, lacrimation, anorexia, diarrhea
Dicoumarol	Entire USA	Common Sweet clover	Penicillum sp Mucor sp Humicolor sp	Hemorrhages due to anti-Vitamin K effect in liver. Abortions in late gestation, lameness, hematomas

depleted. Thus many mycotoxicoses are not apparent until after several days or weeks of exposure. Certain mycotoxins cause profound neurochemical or endocrine effects, altering behavior, appetite and reproductive function. Finally, several major mycotoxins are immunosuppressive and may increase susceptibility to infectious diseases or reduce response to vaccinations.

Metabolism or biotransformation of a mycotoxin can lead either to activation or detoxification, depending on the mycotoxin. Metabolized aflatoxin is more toxic and carcinogenic than the original Aflatoxin B1 while metabolism of the trichothecenes (e.g. T-2 toxin) reduces their toxicity and increases their excretion.

Diagnosis of Mycotoxicoses

Mycotoxicoses are difficult to differentiate clinically from other common disease or management problems and may be occurring concurrently with them. When mycotoxins cause acute disease or large death loss, close evaluation of the clinical history and feed supply are usually necessary to suggest tentative diagnoses. Generally the diagnosis cannot be confirmed without some diagnostic assistance. This is especially true when mycotoxicoses present as chronic conditions related to poor performance, ill thrift or increased susceptibility to infectious disease.

Mycotoxicosis are often sporadic both seasonally and geographically, so epizootiology is often difficult to assess quickly. Due to uneven distribution within grain or feed supplies, the offending agent may have been consumed prior to investigation.

While analysis for major mycotoxins in feed and forages is commonly practiced, detection of toxins in tissues is not routinely available, limiting the confirmation of mycotoxin induced disease. Furthermore, many mycotoxins are rapidly excreted from animals, so lack of consumption for several days prevents residue detection even when analytical tests are available. In some cases, diagnosis may depend on test feeding of the suspected ration with reproduction of the characteristic mycotoxic disease. When test feeding is used, the best approach is to use animals of the type and age affected by the suspect mycotoxin. They should be offered suspect forage for at least two to three weeks to account for the potential delayed effects of the toxins. Test parameters should include feed consumption, weight gain, clinical observation and a minimal clinical chemistry panel for liver and kidney function. In many cases it will be impossible to recreate all the conditions present at the time of a clinical mycotoxicosis.

Sampling and Submitting Feeds for Laboratory Analysis

When feeds or forages are purchased, owners should be advised to retain an identified sample of the feedstuff for up to several months after use has begun. Often when purchased feeds are suspected, there is no remaining representative sample for testing, leaving only the recollection of the parties involved as evidence on which to base a decision.

Samples of feed or forage for analysis or feeding trials should reflect all sources of feed available at the time the problem occurred. Individual plants or seeds can be heavily contaminated with mycotoxins and be responsible for measurable levels of toxin within a large lot of forage or grain. Representative sampling of forages or grain is important since contamination can vary widely among different sites in the same field or supply of stored forage.

Mold activity (even molds known to produce toxins) is no sure indicator of mycotoxicosis. Temperature, substrate, moisture, and number of contaminating molds all may affect toxin production.

For grains, representative samples may be taken from storage units in two ways: (1) stream sampling, where a large quantity of grain is transferred by auger or gravity and periodic samples are taken from the moving stream, combined, mixed and an aliquot submitted for analysis, and (2) probe sampling, where a grain probe is used to select individual aliquots from a large storage bin. These aliquots are combined and thoroughly mixed before submission for analysis.

Forage samples present even greater challenges in sampling because plants are relatively large units, and differing cultural or microenvironmental conditions in a field may affect stress and mycotoxin formation. Sampling pasture forages should be done by establishing a grid throughout the pasture and randomly selecting several samples from each section of the grid. These can be analyzed as a composite, by grid section or as individual samples which appear questionable. Silage can be sampled by an approach similar to grains. Multiple samples can be collected from baled hay using a core sampler.

Samples for mycotoxin analysis may change greatly if not properly preserved and shipped. Grain or forage samples of greater than 15% moisture should be oven dried at 60°C and shipped in paper or cloth bags, or high moisture samples can be frozen and shipped to arrive still frozen. Remember that some molds can grow at refrigerator temperatures, and silage when exposed to air in a plastic bag can mold rapidly. For long term storage, dry the samples to 12% moisture and store in moisture proof containers.

Selection of Laboratory Analyses

Mycotoxins are complex and varied organic chemicals. Only recently have state and private laboratories developed analytical tests that are accurate, precise and sensitive. Often an effective analysis is confined to certain matrices such as corn or feed grains, and when it is applied to forages, the pigments and oils can interfere with the analysis, causing failed detection and/or false positives. With the advent of radioimmunoassay and ELISA technologies, several commercially available test kits have been utilized (Table 2). These are generally only for screening purposes to detect presence of a mycotoxin at levels above a specified detection limit. In some cases, cross reactivity can occur with similar compounds, and quantitation is difficult. They can serve a valuable role in providing rapid high-volume screening, but should not currently be considered as supplanting traditional chemistry methods.

Laboratory determination of mold counts or isolation of molds by genus only is generally not considered adequate diagnostic confirmation of a mycotoxicosis. Mold spores are common in feedstuffs, and only when conditions are proper for their growth and toxin production do they have the potential for harm. When mold populations are mixed and heterogeneous, there may be less probability of mycotoxin formation than when only one toxin producing species and strain are present.

When selecting a laboratory, look for an organization that provides a written schedule of fees and services

and that provides information about their quality control program, the qualifications of their staff, and where they are accredited. For example, state veterinary diagnostic laboratories are accredited by the American Association of Veterinary Laboratory Diagnosticians. In addition, the laboratory should be able to provide instructions in collection and preservation of samples and interpretation of the results in the context of the samples being analyzed and the source of the specimens. Finally, the laboratory should be willing to inform you when your sample is inadequate or improperly submitted.

Table 2. Commercially Available Mycotoxin Test Kits*

Mycotoxin	Name	Methodology	Sources
Aflatoxin	Aflatest	Affinity column	VICAM
	Afla20	ELISA	International Diagnostic
	Agri Screen	ELISA	Neogen Corp.
	Cite-Probe	ELISA	IDEXX
	EZ Screen	ELISA	Environmental Diagnostics
	Total Aflatoxins	Affinity column	BioCode, Ltd.
	Aflatoxin M_1 Test	ELISA	Transia
	Target	Selective Absorption	TerraTek
	HV Mini column	Mini column	Romer Labs, Inc.
DON (Vomitoxin)	Agri Screen	ELISA	Neogen Corp.
	DON		Romer Labs, Inc.
Fumonisins	Agri Screen	ELISA	Neogen Corp.
	Fumonitest	Affinity column	VICAM
Ochratoxin	Ochratest	Affinity column	VICAM
T-2 Toxin	T-2	ELISA	Neogen Corp.
	T-2	Mini column	Romer Labs, Inc.
Zearalenone	Zearalatest	Affinity column	VICAM
	Agri Screen	ELISA	Neogen Corp.
	Zearalenone	Mini column	Romer Labs, Inc.

*Manufacturers and/or sources of commercial mycotoxins

Recognition of Mycotoxin Related Diseases

Diseases caused by mycotoxins may be difficult to recognize, since many are chronic, subtle and of delayed onset. Conversely, they are specific chemicals and usually cause somewhat consistent effects that may be altered by dosage received. Mycotoxic diseases may sometimes be regional and correlated with local weather or cultural conditions, while in other cases poor storage conditions allow for contamination of the feed supply in only one herd. Careful investigation may be necessary to establish a relationship with a specific feedstuff. This is especially difficult, since some effects may not be apparent for several days or weeks after consumption, or they may be delayed and not recognized until the contaminated feed is unavailable to be sampled.

Specific Forage Mycotoxicoses

Several mycotoxins are associated with forages in North America. In some cases they are responsible for widespread disease and economic loss (e.g. fescue toxicosis) while others appear to occur only rarely (e.g. ryegrass staggers). Numerous questions arise about moldy forages with post-harvest mold infestation. Unfortunately little definitive information is available to evaluate the effects of post-harvest fungal infestations to specific livestock problems. In such cases, attention to the general principles of evaluating and managing mycotoxin problems (i.e. careful history and appropriate test feeding) can aid in practical resolution of immediate animal health issues.

The mycotoxins commonly associated with feed grains (e.g. aflatoxin, DON or vomitoxin, zearalenone, ochratoxin or citrinin, and fumonisins) are rarely found in forages, and when reported are generally at relatively low levels. This paper will discuss, for comparative purposes, the common grain mycotoxins. However, their presence in molded forages is not expected unless grain is part of the harvested forages. Examples are ergot infested small grains incorporated in straw, and green chop or silage where the grain mycotoxins are part of the chopped forages. When TMR diets are used, the separation and detection of source of mycotoxin may be difficult.

Major forage related mycotoxicoses in North America include the following:

- **Fescue toxicosis**
- **Ryegrass staggers**
- **Paspalum staggers**
- **Sweet clover disease**
- **Slobbers syndrome (Slaframine toxicosis)**
- **Photosensitization from Pithomyces chartarum**

Fescue toxicosis

Fescue grass (*Festuca arundinaceae* Schreb.), also commonly known as Tall Fescue is a major forage grass over approximately one-third of the USA involving an estimated 35 million acres. It is readily established, and provides good yields and favorable nutrient content while tolerating poor quality soils, drought, heat and insects. Much of the early plantings of fescue ("Kentucky 31") co-existed with a fungus living within the fescue plants. This endophytic fungus, *Acremonium coeniphialum* does not affect appearance of the plant and actually confers on the fescue some beneficial effects such as increased drought resistance and insect tolerance. *Acremonium coenophialum* is considered a mutualistic mycosymbiont and natural agent of biological protection of tall fescue. (Tsai *et al* 1992) The endophyte

is transmitted through infected fescue seed. Recently, endophyte-free strains of fescue have been developed and made commercially available, allowing producers to develop management strategies to alleviate fescue toxicosis.Three major livestock disorders are associated with fescue. These include fescue foot, bovine fat necrosis and fescue toxicosis ("summer syndrome") (Ball *et al*1991).

Fescue foot is a dry gangrene of the extremities, affecting primarily the feet, tail and ears. Severity varies depending on intake of infected forage and weather conditions. Cold weather accentuates the severity of clinical signs and may increase the incidence of peripheral necrosis. Ergopeptide alkaloids are produced in the endophyte-infected plant. Temperature and blood flow in the extremities are reduced, leading first to lameness followed by separation of the hoof at the coronary band and dry gangrene very similar to classical gangrenous ergotism. The toxin(s) associated with endophyte-infected tall fescue reduce blood flow to peripheral and core body tissues, and the effect persists for up to 8 days after removing the toxin(s) from the diet (Rhodes *et al* 1991). Lameness is commonly observed to begin in the rear limbs of cattle. Currently there are no antidotes for fescue foot. Infected pastures are more a problem if stockpiled for winter grazing, and mowing to reduce the accumulation of seed heads seems to reduce incidence and severity of the lesion. Known infected fescue should avoided or limited during cold weather.

Bovine fat necrosis is a condition reported mainly from the Southeastern USA and has been associated with nearly pure stands of fescue that are heavily fertilized with nitrogen fertilizer or poultry litter. The lesion consists of large masses of hardened fat in the abdominal cavity. Necrotic fat is calcified and higher than normal fat in ash, calcium, magnesium and cholesterol. These may cause physical interference with intestinal motility or with calving, but the overall incidencem of economic loss is relatively low.

Fescue toxicosis, also known as **"summer syndrome"** or **"summer slump"** is the most economically damaging of the fescue problems. Animals grazing infected pastures during periods of high ambient temperatures commonly spend less time in daytime grazing and have reduced forage intake resulting in lower weight gains and reduced milk production. In severe cases, agalactia may occur. In addition, signs of heat intolerance include increased respiratory rate, higher body temperatures and more time spent in shade or water. Although heat intolerance is a factor in fescue toxicosis, poor weight gains can occur on endophyte infected pastures during cool weather as well. Some affected animals show increased salivation. Cows have reduced reproductive performance and fertility, indicated by increased postpartum intervals and reduced pregnancy rates.

Laboratory analyses have shown reduced serum prolactin levels. Endophyte toxins may reduce prolactin synthesis and release and may alter activity of dopaminergic neurons (Schillo *et al* 1988). Imbalances of prolactin and melatonin resulting in reduced blood flow to internal organs are reported as potential causes of reduced reproduction, growth, and maturation in livestock consuming endophyte-infected tall fescue (Porter and Thompson, 1992).

Recently, several researchers have shown that ergopeptide alkaloids found in endophyte-infected fescue are associated with the fescue toxicosis syndrome. Ample evidence exists as well that ergot alkaloids affect prolactin release. When total ergovaline consumption per cow was between 4.2 and 6.0 mg/day, decreased performance (body weight) was not fully accounted for by reduced forage intakes, leading to the conclusion that nutrient utilization may be reduced by endophyte-infected fescue (Thompson *et al* 1992[a]). Prolactin is associated with the onset of milk production and increases when environmental temperature is elevated.

Studies on laboratory animals suggest potential immunosuppressive effects of endophyte-infected fescue. Ratshad reduced serum titers to sheep red blood cell (SRBC) and lowered white cell counts. In addition, spleen cells from mice fed endophyte positive diets had reduced response to the mitogens Concanavalin A and lipopolysaccharide (Dew *et al* 1990). These potential immunologic effects in rodents have not been demonstrated in livestock.

Several treatments have been investigated which show promise of reducing the effects of fescue toxicosis. These are reviewed briefly, even though they are currently not approved nor in general clinical use.

Animals treated with metoclopramide grazed approximately four times longer between noon and 4 PM than controls offered the same toxic fescue pastures. Results suggest that dopaminergic processes may be important in fescue toxicosis (Lipham *et al* 1989). Metoclopramide administration also increased serum prolactin in cattle fed endophyte-infected fescue (Thompson *et al* 1992[a]). Alkaloids in ingested endophyte-infected tall fescue appear to induce thiamin deficiencies in cattle that result in symptoms of tall fescue toxicosis (Dougherty *et al* 1991). Thiamin supplementation appears to alleviate some of the effects of grazing on endophyte-infected tall fescue during warm weather (Lauriault *et al* 1990). Steers fed Ammoniated toxic fescue hay had significantly higher serum prolactin concentrations and lower rectal temperatures than steers receiving only endophyte-infested fescue hay (Kerr *et al* 1990). Cimetidine (a histamine H2 receptor antagonist) also lowered body temperatures to control levels by day 4 in sheep fed Endophyte-infected fescue hay (Zanzalari *et al* 1989), although this approach is not

yet clinically practicable. Recently, treatment with ivermectin pouron has increased gains for steers grazed on toxic fescue pastures (Bransby, 1993).

Perennial Ryegrass Staggers

Perennial ryegrass, *Lolium perenne*, has recently been documented in the occurrence of a tremorigenic condition in the Pacific Northwest. The condition, known as ryegrass staggers has long been recognized in Australia, New Zeland and Europe. It usually occurs on intensively grazed pastures during late summer or fall. An endophytic fungus, *Acremonium lolii*, has been associated with the toxic grasses, and the toxins lolitrem-B and tunicamycin have been implicated as the causative agents. Increasing levels of infection are correlated with higher concentrations of the toxins. Concentration of the endophyte and toxin are greatest in the lower leaves and stems of toxic pastures.

Clinical signs of ryegrass staggers occur after one to two weeks of continuous exposure. Morbidity may be high (10-75%) in a herd, but deaths are uncommon if the problem is recognized and animals are removed to uncontaminated pastures. Clinical signs appear related to disturbances in excitatory amino acid neurotransmitters such as GABA. The signs are typical of the tremorgenic mycotoxins, ranging from mild and subtle when animals are at rest to exaggerated and severe when animals are excited or forced to exercise. Signs progress from fine tremors about the head and neck to stiffness, incoordination, hypermetria, tremors, opisthotonus and seizures. If animals are left undisturbed, signs subside in five to ten minutes. Recovery occurs one to three weeks after ingestion of the infected ryegrass stops. Deaths are rare, except from accidental events such as trauma or drowning during the acute seizures. The condition is considered reversible, even though some reports describe permanent neurologic lesions in experimental animals (Nicholson, 1989; Galey, 1991).

Diagnosis of ryegrass staggers has been confirmed by use of mouse bioassay, identification of the endophyte in forage plants, and chemical analysis of plants to detect the tremorgenic toxins. Samples for examination should include the lower portions of stems and leaf sheaths (just above the root) from suspect plants. Other sources of tremorgenic toxins include Dallis grass (*Paspalum dilatatum*) infected with *Claviceps paspali*, Bermuda grass (*Cynodon dactylon*), ergot (*Claviceps purpurea*), white snakeroot (*Eupatorium rugosum*), Ohio buckeye (*Aesculus glabra*), and penitrem A from moldy walnut hulls (*Juglans spp.*). Consideration of the time of year and available exposure to potential tremorgens will usually eliminate many of the differential diagnoses. Insecticides such as chlorinated hydrocarbons, organophosphates and pyrethrins can cause clinical signs similar in some respects to ryegrass staggers, but these chemical causes can be confirmed by analytical testing.

Resolution of ryegrass staggers can be enhanced by removing affected animals from contaminated pastures to a quiet, protected location and supplementing with clean feed. There is no specific antidote. Prevention of future poisoning could include diluting the forage with other hay or grain, avoiding overgrazing, and pasture renovation with endophyte-free strains of perennial rye.

Dallis Grass Staggers (Paspalum Staggers)

Dallis grass staggers, also known as Paspalum staggers, is caused by an ergot (*Claviceps paspali*) of Dallis grass (*Paspalum dilatatum*). The Dallis grass ergot is a pale tan cauliflower shaped sclerotium two to three times the size of the Dallis grass seed. Infection occurs in late summer.

Tremorgenic toxins similar to penitrem A affect mainly cattle, but horses and sheep are also susceptible. Morbidity is high, but mortality is usually less than 10 percent and clinical signs occur from two to three days of exposure to as little as 3 grams of ergot bodies per kilogram body weight. Clinical signs are similar to ryegrass staggers, but seizures may be more severe and affected animals may be belligerent or aggressive (Nicholson, 1989).

Diagnosis of Dallis grass staggers is suggested by observation of the characteristic clinical signs and detection of the typical ergot sclerotia in pasture or hay.

No specific antidote is available for Dallis grass staggers. Animals should be kept quiet and in a protected and secure enclosure. Affected animals recover in a few days if clean feed is given. Preventive measures should include clipping the pasture or close grazing to prevent seed formation which serves as a substrate for the fungus.

Sweet Clover Disease

Both yellow sweet clover (*Melilotus officinalis*) and white sweet clover (*Melilotus alba*) contain coumarin which can be dimerized by fungal activity to form dicoumarol. Dicoumarol is a potent anticoagulant that acts by preventing the reactivation of vitamin K which is necessary to complete the synthesis of factors II, VII, IX and X in the coagulation system. Several general of fungi, including Aspergillus, Penicillium, Humicolor and Mucor have been associated with this activity.

Currently, low coumarin sweet clover varieties are available, but sweet clover is not a commonly utilized forage. It is planted as a soil-building or cover crop where use for hay or pasture is not expected. Exposure to sweet clover may occur as a spurious contaminant of pasture or hay, or when hay is cut from federal set-aside land or

other conservation-based plantings. Silage containing moldy sweet clover is also toxic. The use of large round bales stored outside for long periods of time has been suggested by some as a predisposing factor to molds that could cause sweet clover disease. Dicoumarol concentrations of less than 20 ppm are usually non-toxic, while levels above 40 ppm are considered unsafe. Concentrations greater than 60 ppm experimentally have caused coagulopathy within approximately three weeks of continuous feeding (Casper, 1993).

Clinical effects of dicoumarol toxicosis can range from sudden death due to massive hemorrhaging to chronic bleeding with epistaxis, vaginal bleeding, bloody feces, subcutaneous hemotomata, lameness, and occasionally neurologic signs if bleeding is in the central nervous system or spinal cord. Pregnant cows may abort due to placental bleeding, and calves born to dams fed moldy sweet clover may have coagulopathy at birth. Hematomas usually occur in the submandibular, brisket, flanks, legs and other areas that physically contact feed bunks, fences or buildings. Diagnosis of sweet clover disease is usually suspected when a vitamin K responsive coagulopathy occurs in the presence of moldy sweet clover. Differentiation of sweet clover from alfalfa can be done by examining the tri-lobed leaflets of the plant. Sweet clover has a center leaflet that is more elongated than alfalfa and the distal third of the leaflet margins are serrated. Clinical laboratory findings of anemia, prolonged clotting time and elevated one stage prothrombin time (OSPT) and/or activated partial thromboplastin time (APTT) are adjuncts to diagnosis. Analytical identification of dicoumarol in forages (> 30 ppm) or liver (> 0.5 ppm) will provide confirmation of probable toxic exposure. Live animals may have detectable concentrations of dicoumarol or its metabolites in blood or urine.

Treatment of dicoumarol poisoned animals should begin with immediate administration of Vitamin K1 at 1-3 mg/kg body weight daily, given intramuscularly in divided doses using a small gauge needle. Menadione (vitamin K3) is not effective. In acute emergency situations, administration of three to five liters of blood from a normal animal will provide the coagulation factors needed to save an animal. Animals must be promptly removed from the sweet clover source. Alfalfa hay is a good substitute forage since it is high in natural vitamin K. Coagulation improvement will occur in from 12 to 24 hours after vitamin K injection and affected animals are usually normal in 4 to 7 days. Monitoring of coagulation status and administration of vitamin K for 3 to 5 days is recommended (Casper, 1993).

Sweet clover poisoning is best prevented by feeding only properly cured hay, planting low coumarin cultivars of sweet clover, and avoiding prolonged feeding of known contaminated hay. Feeding sweet clover for one week alternated with alfalfa or other sources for two weeks has prevented sweet clover disease. Menadione has not been effective in preventing sweet clover disease. Sweet clover should not be fed in late gestation or within two weeks before surgical procedures or other events that cause trauma and potential bleeding.

Slobbers Syndrome (Slaframine toxicosis)

The conditions commonly known as "Slobbers syndrome" or "salivary syndrome" is associated mainly with red clover (*Trifolium pratense*) infected with the fungus *Rhizoctonia leguminocola* (Black patch disease). Contamination is most common in wet cool years and in stands of nearly pure red clover, although other legumes (white clover, alsike alfalfa,and others). The fungus can overwinter in infested plants or survive up to two years in seed. Properly dried hay will remain contaminated and fungal activity can continue development in baled hay. An indolizidine alkaloid known as slaframine is the toxic principle (Smalley and Sanderson, 1993).

Horses appear to be most susceptible, but clinical effects are documented in cattle. Animals may be affected within one hour after consuming contaminated hay. There is profuse salivation early and this may continue for 24 to 72 hours. Other concurrent signs include mild lacrimation, frequent urination and diarrhea. Bloat can occur and most affected animals have partial to complete anorexia.

In addition to stimulation of salivation, slaframine causes increased pancreatic fluid secretion, bradycardia and bradypnea, hypothermia and increased intestinal and uterine motility.

Morbidity may be high, but mortality generally is low, and prompt removal of contaminated hay is followed by recovery in 24 to 48 hours. Presence of other alkaloids, especially swainsonine, has been suggested to complicate the clinical response and introduce neurologic disturbances as well. The real role of swainsonine in the salivary syndrome is not clear.

Diagnosis is usually based on recognition of the characteristic excessive salivation and other clinical effects correlated with a history of consumption of red clover or other legumes. Identification of the lesions or black patch disease or isolation of the fungus further supports a presumptive diagnosis. If needed, chemical analysis for slaframine and swainsonine is available through diagnostic laboratories.

There is no specific antidote to slaframine toxicosis, although atropine may control at least some of the prominent salivary and gastrointestinal signs. Removal of animals from the contaminated hay is essential.

Prevention of black patch disease has been difficult by chemical treatment of the plants themselves. Seed treatment may eliminate the infection for new seeding, and producers can check with seed suppliers for

varieties that are relatively resistant to black patch. Avoiding use of pure red clover for forages or using it as part of a forage mixture is helpful, and the low incidence of the salivary syndrome is probably due in part to the relatively infrequent use of red clover in modern forage systems.

Pithomyces chartarum Photosensitization

Pithomyces chartarum is a fungal saprophyte of a variety of grasses, legumes and weeds. Historically it has been a clinical problem in Australia, New Zealand and South Africa, but is also known to occur in the United States and Canada. Favorable conditions of moisture and temperature promote the growth of *P. chartarum* which can produce a hepatotoxic mycotoxin known as sporodesmin which is concentrated in the spores. Sporodesmin is a biliary system toxin, affecting bile duct epithelium with swelling and obliterative cholangitis. As a result of these cholestatic effects, plant chlorophyll metabolites (phylloerythrins) are not excreted in the bile. They accumulate in blood, and where skin is exposed to sunlight their photodynamic effects induce photosensitization. While sporodesmin has not been definitively linked to naturally occurring photosensitization in North America, the presence of the fungus and conditions that support sporodesmin growth make it a potential problem (Bagley and Shupe, 1986).

The clinical effects of sporodesmin are similar to other photosensitization reactions, but are dose dependent and at high dosages significant mortality may occur. Clinical signs may occur until several days of weeks after initial exposure when representative infected forage is no longer available.

Diagnosis of sporodesmin toxicosis would be enhanced by detection of the liver lesions that include bile duct edema and luminal occlusion accompanied in advanced cased by proliferation of bile ductules and fibrous connective tissue. Clinical laboratory determination of elevated serum bilirubin, gamma glutamyl transpeptidase and aspartate amino transferase would be consistent with sporodesmin toxicosis. Definitive chemical testing is not routinely available, but isolation of the fungus could help in establishing a presumptive diagnosis, although as with other mycotic infections of plants, the fungus does not always produce toxin.

Prevention and control of sporodesmin toxicosis is enhanced by grazing or haying management which reduces the amount of dead plant litter. This is usually accomplished by well controlled rotation grazing. In high humidity areas (e.g. New Zealand) pastures are sprayed with fungicides such as Thiabendazole or Benlate to suppress sporulation during periods of high risk. Zinc supplementation at 20 mg/kg body weight is protective against liver damage from sporodesmin, but administration at this level has been logistically difficult.

Major Mycotoxicoses From Feed Grains in North America:

- **Aflatoxin**
- **Ergot**
- **Trichothecenes**
- **Zearalenone**

There is little evidence that these mycotoxins occur with significant incidence or at clinically relevant levels on forages in North America. Their significance to ruminants is reviewed briefly for comparative purposes and because they may be economically more important than the recognized forage mycotoxins just discussed.

Aflatoxin

Cattle, sheep, and other ruminants are less susceptible than monogastric animals or poultry. Calves are more susceptible than mature animals. Dietary concentrations as low as 200 ppb fed to calves has been associated with reduced growth and liver damage.

Concentrations of 2.2 ppm fed for sixteen weeks results in death of weanling calves. Concentrations of 1-2 ppm in mature cattle for short periods of time results in reduced gain and decreased milk production. Abortion and infertility are not generally expected.

Major clinical effects include depression, anorexia, reduced gain or milk production, subnormal body temperature and dry muzzle. Lesions of liver damage range from acute to chronic. Aflatoxin has been reported to decrease cellulose digestion, reduce volatile fatty acid formation and inhibit proteolysis in artificial rumen systems. Aflatoxin at high concentrations (1-4 ppm) can reduce rumen motility.

Although estimates vary, Aflatoxin in milk appears at approximately 1% of the dietary concentration (Range 0.2-3.2%). Most Aflatoxin is excreted within 72-96 hours after exposure stops. Dairy cattle should not be fed feed containing more than 20 ppb Aflatoxin. This should prevent the occurrence of milk residues where the current action level is 0.5 ppb. Liver and kidney appear to retain measurable residues of Aflatoxin longer than other tissues (but less than 2 weeks).

Acute disease, is characterized by depression, anorexia, icterus, and hemorrhages. Liver function tests and microscopic evaluation of liver should be used as adjuncts to support clinical diagnosis. Aflatoxin is readily detected in feed supplied at levels far below those necessary to produce clinical poisoning. Urine, milk, and blood may contain detectable residues during acute exposure, and urine concentrations of Aflatoxin M1 may persist for up to a week after exposure to high levels stops.

Mold inhibitors may aid in preventing further growth and toxin formation of A. flavus in prepared

feeds, but will not destroy preformed toxins. Treatment with anhydrous ammonia for 10-14 days reduces Aflatoxin in grain, but due to uncertainty of the breakdown products, FDA has not cleared this detoxication method. Hydrated sodium calcium aluminosilicate (HSCAS) reduced but did not eliminate residues of Aflatoxin M1 in milk of dairy cows fed Aflatoxin B1. HSCAS has high affinity for Aflatoxin and can reduce absorption of Aflatoxin from the gastrointestinal tract.

Action levels recently suggested by FDA are:

Human Food and Milk	0.5 ppb
Corn of Unknown Destination	20 ppb
Young Animals	20 ppb
Dairy Cattle	20 ppb
Breeding Cattle	100 ppb
Finishing Cattle	300 ppb

Ergot

Ergot alkaloids are produced by sclerotia of *Claviceps purpurea* and invade the ovary (seed) of grass flowers where they appear as dark brown to black banana shaped sclerotia. Recently Tall Fescue (*Festuca arundinaceae*) invaded by the endophytic fungus *Acremonium Coeniphialum* has also been shown to contain the ergot alkaloid ergovaline (see Fescue).

Ergot alkaloids may induce uterine contractions in a sensitized uterus, but solid evidence for abortion is limited. High concentrations of ergot alkaloids cause peripheral vasoconstriction and produce classical dry gangrene of extremities (feet, tail, ears) that is exacerbated in cold weather.

Ergot alkaloids inhibit prolactin release and this prevents mammary development in late gestation as well as reducing or eliminating milk secretion. The result is agalactia at parturition. The agalactia is reversible five to seven days after ergot is removed from the diet.

Ergot alkaloids may also be responsible for heat intolerance in cattle when consumption occurs in warm weather. This association of ergopeptide alkaloids with heat intolerance and prolactin suppression is well established with endophyte infected fescue. Such animals have elevated rectal temperature, seek shade and/or water holes, eat less and are often depressed.

Trichothecene Mycotoxins

These include over 60 metabolites produced by *Fusarium sporotrichioides* or *F. roseum.* Best known are T-2 toxin, diacetoxyscirpenol and vomitoxin (DON). DON is by far the most commonly reported. They are almost always reported from feed grains. Cool, wet weather during maturation of grain or harvest favor production of trichothecenes. They have commonly been reported on grain overwintered in the field. Reports of occurrence on forages have been in past years, and recent techniques have not revealed a significant contamination problem on forages.

Except for vomitoxin, effects described, for trichothecenes are mostly from dosing experiments. Most animals refuse to consume high concentrations of trichothecenes and never achieve the full range of reported toxic effects. Forced intake of trichothecenes can cause anorexia, enteritis, lymphoid depletion, leucopenia, bone marrow depression, coagulopathy, and suppression of cell mediated immunity. Prolonged dermal contact with T-2 and some other trichothecenes can cause dermal irritation and necrosis.

Cattle begin to refuse vomitoxin (DON) contaminated feed at approximately 10 ppm. Limited studies in dairy cows indicate six ppm may be a refusal level. Recently, some investigators have associated presence of low levels of vomitoxin in feed with reduced milk production. DON itself may not be responsible, but is considered by some to be a "marker" toxin indicating potential adverse effects. More studies are needed, and so far a cause-effect relationship has not been established.

Trichothecenes are rapidly metabolized both by rumen microorganisms and systemic enzymes. Degradation products are rapidly eliminated and persistent residues are not expected. Very little DON (vomitoxin) is excreted in milk (Beasley, 1993).

Zearalenone

This mycotoxin is an estrogen but less potent estrogen than estradiol or DES. In the USA, grain rarely exceeds 3 ppm. Most common sources are corn and wheat during wet, cool seasons that cause wheat scab or pink rot of corn. It has been very rarely reported in hay, and usually at concentrations not known to cause toxicosis in cattle. Remember that other plants (e.g. legumes) contain estrogens which could mimic some effects of zearalenone. Zearalenone is stable to drying, but the effect of ensiling on zearalenone is not known.

Dairy heifers may have increased infertility and reduced conception at zearalenone feed concentrations above 12 ppm. Mature cows are affected at concentrations above 25 ppm. Some reports indicate vaginitis and vaginal secretion may occur. Virgin heifers may have mammary enlargement. Small amounts of zearalenone may be passed in milk, but residues are not believed to persist for more than a few days once exposure is stopped (Diekman and Green, 1992).

Alfalfa and alfalfa meal in some species will reduce absorption and increase fecal excretion of zearalenone. Whether this occurs in dairy cattle has not been determined.

Guidelines for Prevention and Management of Mycotoxicoses:

1. The best management is to avoid use of known moldy grain or forages in dairy cattle rations. Mycotoxins may occur for which there are no documented effects nor means of identification.
2. Dilution of contaminated feedstuffs will reduce the initial mycotoxin level. Remember that molds present may contaminate "clean" feed or forage if storage conditions are not optimal.
3. Many mycotoxins in grain are associated with low quality, small, cracked or broken seeds. Cleaning of grain will often markedly reduce the mycotoxin load.
4. Heavily molded feedstuffs may lack normal energy and vitamin levels. Supplementation, after nutritional analysis, may be helpful.
5. Testing of suspect molded forages and grains may be helpful to eliminate presence of known mycotoxins, but will not establish complete safety. Use of mold counts or spore counts are not a definitive means to establish safety.
6. Storage should be at 13-15% moisture to prevent recontamination by fungal activity.
7. Organic acids will prevent mold growth, but not destroy preformed toxins. Some adsorbents, such as aluminosilicates are useful to alleviate aflatoxin effects, but have not been effective for other mycotoxins.
8. Storage areas and feeders should be kept dry and cleaned periodically.
9. For most mycotoxins, there is no specific treament orantidote. Good supplemental nutrition and alternative feed supplies are essential.
10. Supplementation of vitamins and selenium may be helpful, and provision of adequate high quality protein is advisable after animals have consumed mycotoxins.

Manufacturers and/or sources of commercial mycotoxins.

BioCode, Ltd.
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Hesslington
York Y0L 5DE
United Kingdom
+44 904 430 616
Fax: +44 904 430 495

Transia
8, rue Saint-Jeau-de-Dien
69007 Lyon, France
+33 72 73 03 81

TerraTek
400 Wakara Way
Salt Lake City, UT 84108
(800) 372-2522

Romer Labs, Inc.
P.O. Box 2095
Washington, MO 63090
(314) 239-3009

VICAM
29 Mystic Avenue
Somerville, MA 02145
(800) 338-4381

Neogen Corp.
620 Lesher Place
Lansing, MI 48912
(517) 372-9200

International Diagnostic System Corp.
P.O. Box 799
St. Joseph, MI 49085
(616) 983-3122

Environmental Diagnostic Systems Corp.
P.O. Box 908
2990 Anthony Road
Burlington, NC 27215
(800) 334-1116

IDEXX
100 Fore Street
Portland, ME 04101
(800) 548-8733

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