Prevention, Assessment, and Mitigation of Mycotoxicosis in Dairy Cattle

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Summary

Mold growth is an inevitable consequence of feed production, as a result their harmful metabolites "The Mycotoxins" are commonly found in livestock diets. In the last 40 years, great advances in the field of mycotoxins have increased our knowledge on the detrimental effects of these toxins on animal production. Climate change and agronomic practices play an important role in the unpredictability of mycotoxin contamination of feedstuffs. The primary classes of mycotoxins are aflatoxins, zearalenone (**ZEA**), trichothecenes, fumonisins, ochratoxins (**OTA**) and the ergot alkaloids. Due to the high variety of feedstuff utilized in dairy operations and the high production stress typically associated with modern dairying, mycotoxins are important anti-nutritional factors in dairy nutrition programs. In order to maximize dairy performance and health, mycotoxin analysis and prevention strategies must be part of the all dairy nutritional and health programs.

Introduction

Dairy profitability is highly dependent on proper nutrition and health. It is therefore imperative that dairy owners, manager, nutritionists, and veterinarians consider the negative role of anti-nutritional compounds naturally present in feedstuffs commonly utilized to feed these animals. Among these compounds "the mycotoxins", which are toxic secondary metabolites produced by fungi (molds), should be closely monitored and minimized. There are hundreds of mycotoxins known, but few have been extensively researched and even fewer have good methods of analysis that are commercially available. The primary classes of mycotoxins are aflatoxins of which aflatoxin B1 (**AFB1**) is the most prevalent, zearalenone (**ZEA**), trichothecenes - primarily deoxynivalenol (**DON**) and T-2 toxin (T-2) - fumonisins, ochratoxins (**OTA**) and the ergot alkaloids.

A practical definition of a mycotoxin is a fungal metabolite that causes an undesirable effect when animals or humans are exposed. Usually, exposure is through consumption of contaminated feedstuffs or foods. Mycotoxicoses are diseases caused by exposure to foods or feeds contaminated with mycotoxins (Nelson et al., 1993). Mycotoxins exhibit a variety of biological effects in animals: liver and kidney toxicity, central nervous system effects and estrogenic effects, to name a few. Some mycotoxins, i.e., aflatoxin, fumonisin and ochratoxin, are carcinogenic.

Molds, Plants, and Climate Interactions

The primary mycotoxin-producing fungal genera are *Aspergillus*, *Fusarium* and *Penicillium*. Many species of these fungi

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produce mycotoxins in feedstuffs. Molds can grow and mycotoxins can be produced pre-harvest or during storage, transport, processing or feeding. Mold growth and mycotoxin production are related to plant stress caused by weather extremes, to insect damage, to inadequate storage practices and to faulty feeding conditions. In general, environmental conditions — heat, water and insect damage — cause stress and predispose plants in the field or feed in transit or storage to mold growth and mycotoxin contamination (Coulumbe, 1993). Computer models to predict mycotoxin concentrations in corn prior to harvest are based on temperature, rainfall and insect pressure (Dowd, 2004) and similarly for DON in wheat (Prandini et al., 2009). Molds grow over a temperature range of 10 to 40°C (50 to 104°F), a pH range of 4 to 8, aw (water activity) above 0.7 and moisture content >13 to 15%. Most molds are aerobic, and therefore, high-moisture concentrations that exclude adequate oxygen can prevent mold growth. However, in practical situations, molds will grow in wet feeds, such as silage or wet byproducts, when oxygen is available.

Worldwide, approximately 25% of crops are affected by mycotoxins annually (CAST, 1989), which could lead to billions of dollars of losses. The annual economic cost of mycotoxins to the U.S. agricultural economy is estimated to average $1.4 billion (CAST, 2003). Economic losses are due to effects on livestock productivity, crop losses and the costs of regulatory programs directed toward mycotoxins. The implications of mycotoxins on agricultural trade have been reviewed (Dohlman, 2003).

Occurrence and concentrations of mycotoxins are variable by year and associated with variation in weather conditions and plant stresses known to affect mycotoxin formation (Coulumbe, 1993). In the 2009 to 10 crop year, several regions of the U.S. experienced higher concentrations and incidence of mycotoxins primarily due to a wet and delayed harvest season. These weather/climate trends have been more and more frequent in recent years. Climate change and agronomic practices play a critical role in the plant/mold interactions necessary for mycotoxin outbreaks. A recent study by a group of subject matter experts (Wu et al., 2011) hypothesized that climate change (and the overall temperature increase) would play a significant role in increasing aflatoxin and fumonisin contamination in maize, while DON concentrations would see a reduction related to the ambient temperature/mold relationship. However, these researchers postulated that DON concentrations in maize could also increase in relation to climate change related cropping practices and other agronomic changes. One of the most significant and potentially detrimental changes could be the trend to reduce or even eliminate tilling practices. Mansfield et al. (2005) looked at the effect of tilling on DON content in maize and concluded that although tillage type (no-till vs. moldboard till) had no effect on DON incidence, no tilling resulted in significantly higher DON concentrations than moldboard tilling.

Although mycotoxins occur frequently in a variety of feedstuffs and are routinely fed to animals, it is less frequent that mycotoxins occur at concentrations high enough to cause immediate and dramatic losses in animal health and performance. However, mycotoxins at low levels interact with other stressors to cause subclinical losses in performance, increases in incidence of disease and reduced reproductive performance. To the animal producer, these subclinical losses are of greater economic importance than losses from acute effects and even more difficult to diagnose.

Mycotoxicosis

The study of mycotoxins began in early 1960's with the outbreak of Turkey-X disease in the U.K. This outbreak was linked to peanut meal imported from Brazil (Sargeant et al., 1961). Because of an intensive multidisciplinary research effort, a blue-fluorescent toxin was isolated and mycelia of *A. flavus* were observed. *A. flavus* was shown to produce the same toxic compound(s) found in the toxic peanut meal. The toxin was characterized chemically and biologically and was given the trivial name aflatoxin. Aflatoxin was shown to be very toxic and carcinogenic in some of the test animal species used, and it resulted in a toxic metabolite in milk of dairy cows (Allcroft and Carnaghan, 1962; 1963).

The discovery of aflatoxin and elucidation of some of its effects led to research on other livestock health and production problems linked with moldy feedstuffs. This research led to the discovery of additional mycotoxins produced by other fungi. In dairy cattle, swine and poultry, mycotoxin contamination of feeds affects growth, milk production, egg production, reproduction and immunity (Diekman and Green, 1992). Mycotoxins have also been involved in outbreaks of human diseases (CAST, 1989).

Animals experiencing a mycotoxicosis may exhibit a few or many of a variety of symptoms, including: digestive disorders, reduced feed consumption, unthriftiness, rough hair coat or abnormal feathering, undernourished appearance, low production, poor production efficiency, impaired reproduction and/or a mixed infectious disease profile. Mycotoxins can increase incidence of disease and reduce production efficiency. Some of the symptoms observed with a mycotoxicosis may therefore be secondary, resulting from an opportunistic disease, present because of mycotoxin-induced immune suppression. Immunotoxic effects of mycotoxins have been reviewed (Bondy and Pestka, 2000; Oswald et al., 2005). The progression and diversity of symptoms in a mycotoxicosis can be confusing, making diagnosis difficult (Schiefer, 1990). Diagnosis is further complicated by limited research, lack of feed analyses, nonspecific symptoms, few definitive biomarkers and interactions with other stress factors.

With few exceptions, a definitive diagnosis of a mycotoxicosis cannot be made directly from symptoms, specific tissue damage or even feed analyses. However, experience with mycotoxin- affected herds increases the probability of recognizing a mycotoxicosis. A process of elimination of other factors, coupled with feed analyses and responses to treatments can help identify a mycotoxicosis. More definitive diagnoses can be made for specific mycotoxins by detecting aflatoxin in milk or for fumonisin by induced changes in sphingolipid concentrations (Riley and Pestka, 2005). Regardless of the difficulty of diagnosis, mycotoxins should be considered as a possible cause of production and health problems when appropriate symptoms exist and problems are not attributable to other typical causes (Schiefer, 1990).

Safe Levels of Mycotoxins

Some of the same factors that make diagnosis difficult also contribute to the difficulty of establishing levels of safety. These include lack of research, sensitivity differences of animal species, imprecision in sampling and analysis, the large number of potential mycotoxins, interactions among mycotoxins and interactions with stress factors (Schaeffer and Hamilton, 1991). Field toxicities appear to be more severe than predicted from laboratory research.

Naturally contaminated feeds are more toxic than feeds with the same level of a pure mycotoxin supplemented into the diet. Aflatoxin produced from culture was more toxic to dairy cattle than pure aflatoxin added to diets (Applebaum et al., 1982). In swine, Foster et al. (1986) demonstrated that a diet containing pure added DON was less toxic than diets with similar concentrations of DON supplied from naturally contaminated feeds. Smith and MacDonald (1991) have suggested that fusaric acid, produced by many species of *Fusarium*, occurs along with DON to produce more severe symptoms. Lillehoj and Ceigler (1975) gave an example where penicillic acid and citrinin were innocuous in laboratory animals when administered alone but were 100% lethal when given in combination. These studies strongly suggest the presence of other unidentified mycotoxins in naturally contaminated feeds and that mycotoxin interactions are extremely important. It is well documented that several mycotoxins may be found in the same feed (Hagler et al., 1984). Abbas et al. (1989) demonstrated *Fusarium* species isolated from Minnesota corn produced multiple mycotoxins. Because animals are fed a blend of feedstuffs and because molds produce an array of mycotoxins, many mycotoxin interactions are possible. Speijers and Speijers (2004) discussed the combined toxicity of mycotoxins; and therefore, suggest daily tolerable intake limits for groups of mycotoxins.

Mycotoxin interactions with other factors also make it difficult to determine safe levels of individual mycotoxins. Animals under environmental or production stress may show the more pronounced symptoms. For example, there is a clear temperature interaction with fescue (ergot) toxicity, such that more pronounced symptoms are expressed during heat stress (Bacon, 1995). Jones et al. (1982) demonstrated that productivity losses in commercial broiler operations occurred when aflatoxin concentrations were below concern levels determined by controlled research in laboratory situations. The researchers hypothesized that general production stress had a significant contribution to the animal's susceptibility to the low concentrations of the toxins. The known dietary factors that interact with mycotoxins include nutrients such as fat, protein, fiber, vitamins and minerals (Brucato et al., 1986; Galvano et al., 2001). Thus, many factors and interactions make it difficult to relate field observations to those from controlled research. Mycotoxin effects vary by species and are also moderated by factors such as sex, age, duration of exposure and stresses of the environment and production.

Overall health and immune status also affect the animal's capability to cope with a specific concentration of a toxin or a combination of toxins. This is primarily due to the many mycotoxins with immunosuppressive properties and their interaction with animal health (Schiefer, 1990). Diagnosis therefore is quite difficult since disease outbreaks may be secondary, resulting from an opportunistic disease, due to a mycotoxin-induced immune suppression. Immunotoxic effects of mycotoxins are reviewed (Oswald et al., 2005; Bondy and Pestka, 2008).

Mycotoxins in Forages

One of the primary differences in exposure between ruminants and monogastrics is related to the role of forages as a source of mycotoxin exposure. Mycotoxins found in forages result in exposure of herbivores to a broad array of multiple mycotoxins. Many different mycotoxins have been found to occur in forages either in the field or in storage as hay or silage (Lacey, 1991). Some mycotoxicoses in cattle resulting from contaminated forages (Lacey,

1991; Gotlieb, 1997; Scudamore and Livesay, 1998) and byproduct feeds (Lillehoj et al., 1991) have been reviewed. Mold grows in hay stored too wet or with damp spots. The limiting factors for mold growth in silage are pH and oxygen. Silages stored too dry or insufficiently packed and covered can allow air infiltration, resulting in growth of yeast, depletion of silage acids, an increase in pH, and thus, conditions conducive for mold growth and deterioration of the silage. The occurrence, prevention and remediation of mycotoxin producing fungi in silage has been recently reviewed by Wambacq et al. (2016).

The most important pasture-induced toxicosis in the U.S. is tall-fescue toxicosis caused by endophytic alkaloids (Bacon, 1995). Other forage toxicoses of fungal origin include ergotism, perennial ryegrass staggers, slobbers syndrome, a hemorrhagic disease associated with dicoumarol produced in fungal-infected sweet clover and sweet vernal grass and syndromes of unthriftiness and impaired reproduction associated with Fusarium (Cheeke, 1995).

In Pennsylvania, Mansfield and Kuldau (2007) found multiple mycotoxigenic molds, including Aspergillus, Fusarium, Penicillium and Alternaria, in corn silage samples at harvest and after ensiling, suggesting the possible presence of multiple mycotoxins. El-Shanwany et al. (2005) isolated 43 fungal species belonging to 17 genera from 40 silage samples collected in Egypt. The most prevalent genera were Aspergillus and Penicillium followed by Fusarium and Gibberella. Mycotoxins were found in 206 of 233 grass or corn silage samples collected in Germany during 1997-1998 (Schneweis et al., 2000). Penicillium was the dominant genus followed by Mucoraceae, Monascus and Aspergillus. Penicillium is a major silage mold and may be a greater silage problem because it grows at a lower pH than do other molds.

Mansfield et al., (2008) investigated the presence of four Penicillium-produced mycotoxins (roquefortine C, MPA, patulin and cyclopiazonic acid) in fresh and ensiled corn silage in Pennsylvania. The four mycotoxins were often found to co-contaminate freshly harvested corn and were generally found in greater frequencies and concentrations after ensiling. Auerbach et al. (1998) found P. roquefortii in 89% of visibly moldy forage samples and 85% of samples without visible mold. Surveys of grass and corn silages in Europe have found an occurrence of P. roquefortii in as many as 40% of samples (Auerbach, 2003). Penicillium-produced mycotoxins in silages, such as roquefortine C, MPA and PR toxins, have been associated with herd health problems (Auerbach et al., 1998; Seglar et al., 1999; Sumarah et al., 2005). Data from Boysen et al. (2000), Seglar et al. (1999) and Sumarah et al. (2005) point to the possibility that PR toxin is a silage mycotoxin of potential concern. Seglar et al. (1999) suggested that PR toxin is a good marker for silages associated with dairy herds with health problems.

Mycotoxin Testing

The accurate determination of mycotoxin concentrations in grain and feeds depends on accuracy from sampling to analytical techniques. A statistically valid sample must be drawn from the lot, which is not simple because mycotoxins are distributed unevenly in grains and other feedstuffs. Most of the error in a single analysis is due to sampling — as much as 90% of the error is associated with the taking of the initial sample (Whittaker, 2003). Once collected, samples should be handled to prevent further mold growth. Wet samples may be frozen or dried before shipment, and transit time should be minimized.

The second-largest source of error is inaccurate grinding and subsampling of the original sample. Finally, the subsample is extracted, the extract purified using one of several techniques, and then the toxin is measured. Toxin determination may be by thin-layer chromatography plates, high-performance liquid chromatography, gas-liquid chromatography, enzyme-linked immunosorbent assays, spectrophotometer or by other techniques. New technologies are progressing rapidly.

Mold spore counts may not be very useful and are only a gross indication of the potential for toxicity, but mold identification can be useful to suggest which mycotoxins may be present. Blacklighting for bright-greenish-yellow flourescence (**BGYF**) is often used as a screening technique for aflatoxin in corn, but it is very inaccurate. Newer and better methods should be used.

Generally, laboratories provide analysis for only a limited number of mycotoxins, perhaps including aflatoxin, OTA, DON, ZEA, fumonisin and T-2 toxin. Laboratory analysis may be directed toward detection of high levels of mycotoxins associated with acute toxicity and serious animal disease rather than low levels associated with chronic effects, such as production losses, impaired immunity and significant economic losses. Therefore, minimum detection limits set by a laboratory may inhibit the diagnosis of a chronic mycotoxicosis.

Analytical techniques for mycotoxins are improving, costs are decreasing and several commercial laboratories are available that provide screens for an array of mycotoxins. The Federal Grain Inspection Service (USDA-GIPSA) provides a list on the internet of approved mycotoxin tests for grains and provides excellent background materials for the feed industry (at www.usda.gov/gipsa/pubs/mycobook.pdf). Laboratory methods can be found in "Official Methods of Analysis of AOAC International". Krska et al. (2008) provided an update on mycotoxin analysis focusing on recent developments including multi-mycotoxin methods and quick tests. Maragos and Busman (2010) reviewed the rapid and advanced tools for mycotoxin analysis.

Because analytical methods can be either qualitative or quantitative, done by inexpensive kits or by sophisticated analytical instruments and can be quick or fairly time consuming, it may be difficult to determine and select the right method for the right need (Scudamore, 2005).

Conclusions

More information is needed about why mycotoxins occur, when to expect them, how to prevent their occurrence and how to deal with their presence. More data are needed about animal toxicity and about interactions with other mycotoxins, nutrients and stress factors, such as disease organisms or environmental stress and about the role of mycotoxins in immunosuppression. Improved screening techniques are needed for monitoring mycotoxin occurrence, including the detection of multiple toxins, diagnosing toxicities and prevention and treatment (CAST, 2003).

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