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Mycotoxicoses in Dairy Cattle: A Review

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ABSTRACT

Mycotoxins are toxic secondary metabolites produced by filamentous fungi growing on several agricultural products in various stages of production, from planting to transportation and storage. Diseases caused by mycotoxins are called mycotoxicoses and can affect several animal species, including ruminants. Mycotoxin contamination of dairy cattle diets is also a serious concern for human health, since some mycotoxin metabolites may be excreted in milk, especially the aflatoxin M_1. This study presents a review of current knowledge on the main mycotoxicoses affecting dairy cattle, especially those caused by mycotoxins produced by species of *Aspergillus*, *Fusarium* and *Penicillium*. The main toxic effects of aflatoxins, fumonisins and trichothecenes in dairy cows are presented, as well as the occurrence of toxigenic fungi and their respective mycotoxins in feed and silage for dairy cattle. A comprehensive description of prevention and decontamination strategies for mycotoxins in dairy feeds is also presented.

Key words: Aflatoxins, fumonisins, zearalenone, trichothecenes, occurrence, toxicity

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi that grow naturally in several agricultural commodities, causing a wide range of toxic effects in vertebrates (Whitlow *et al*., 2010). Exposure to toxins predominantly occurs by ingestion of contaminated foods, mainly cereals such as corn, wheat, sorghum, as well as silages produced from these cereals (Alonso *et al*., 2013). The main toxigenic fungi species belong to the genus *Aspergillus*, *Fusarium* and *Penicillium*, which may grow in a wide range of environmental conditions and virtually in any type of food substrate. They can develop in temperatures from 10-40°C and at water activity higher than 0.7 and their optimum pH values range from 4-8 (Bhat *et al*., 2010). The occurrence of toxigenic fungi species and the consequent production of mycotoxins are directly related with the environmental conditions. Species in the genus *Aspergillus* spp. mainly grow in hot climates, whereas most species of *Fusarium* and *Penicillium* spp. prefer milder climates (De Oliveira *et al*., 2014). Simultaneous occurrence of two or more mycotoxins is also possible and may lead to additive or synergistic toxic effects on the susceptible organism.

Toxigenic fungi may contaminate foodstuffs in the different stages of production and transformation, from growth to transportation and storage and may be divided into two main groups: (a) Field fungi, including *Fusarium graminearum* and *F. verticillioides* species, (b) Storage fungi, such as *Penicillium verrucosum* and *Aspergillus flavus*. According to Cigic and Prosen (2009) the most important mycotoxins found in forage and grains are produced by these fungi species, such as deoxynivalenol and nivalenol (*F. graminearum*), fumonisins (*F. verticillioides*), aflatoxins (*A. flavus*) and ochratoxins (*P. verrucosum*). Moreover, mycotoxins generally present great physical and chemical stability, remaining in the foodstuff even after fungi have been removed by routine processing and packaging methods.

The presence of fungi in feed ingredients may cause general, unspecific problems for the animal production, such as reduced feed intake or feed acceptability. However, diseases caused by mycotoxins, known as mycotoxicoses, are the most important health problems caused by fungi contamination. They are characterized by diffuse syndromes, but with predominance of lesions in organs and tissues, such as liver, kidneys, epithelial and central nervous system, depending on the type of toxin. Thus mycotoxicoses can cause significant losses to the animal industry worldwide.

The objective of this review is to describe the occurrence and main effects of mycotoxins found in dairy cattle diets, including problems related to reduced milk yield and contamination of milk products, as well as the techniques currently available to prevent and reduce contamination by toxigenic fungi and mycotoxins.

NUTRITIONAL ASPECTS AND OCCURRENCE OF TOXIGENIC FUNGI IN DAIRY CATTLE DIETS

Nutrition is one of the most important factors in dairy production, representing the main cost in this activity. Nutrient requirements of dairy cows vary according to the stage of pregnancy and lactation. Five distinct feeding phases were determined for optimum production, reproduction and health of dairy cows: Early lactation; 0-70 days postpartum (peak milk production), peak dry matter intake; 70-140 days postpartum (declining milk production); mid and late lactation; 140-305 days postpartum (declining milk production); dry period; 60-14 days before the next lactation/calving; transition period; 14 days before calving. The nutrients required by dairy cows are energy, fiber, protein, water, vitamins and minerals. Pasture provides a balanced source of feed, but is not enough to maintain high-producing dairy cows. Energy is the decisive factor in milk production: it determines milk yield and composition, as well as body weight. The main energy sources in dairy cow feed are carbohydrates and fiber (corn silage, ground corn, whole cottonseed, high moisture shelled corn, corn gluten meal, soybean hulls and meal). Protein and fats in feeds can also be used as energy sources.

Dairy cattle feed may be naturally and simultaneously contaminated by several fungi that are able to produce different toxins. Most of the silage is made up from annual crops. Therefore, mycotoxin concentration in the feed may vary from year to year (Alonso *et al*., 2013). Several species in the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Trichoderma*, *Claviceps*, *Mucor* and *Geotrichum* make up the storage microbiota and are responsible for the production of mycotoxins found in grains and forage (Driehuis, 2013). The problem of food and feed contamination with mycotoxin has attracted great attention of the scientific community in the last decades, given the high risks to human and animal health and the negative economic impact. Studies have demonstrated the incidence of fungi and mycotoxins in several foodstuffs and silage (Alonso *et al*., 2013), as shown in Table 1.

Dairy cattle feedstuffs are often classified as forages and concentrates, but these divisions are not always clearly definable. Concentrates usually mean high-quality, low-fiber feeds and include cereal grains, milling by-products, protein sources and fats. Therefore, concentrates are more susceptible to mycotoxin contamination. Energy is mostly derived from starches, sugars, other readily available carbohydrates, as well as fats and oils. Forages are characterized by being more

Table 1: Main fungi and mycotoxins found in animal foodstuffs and silage

Fungi	Mycotoxins	Types of foodstuffs	References
Aspergillus	Aflatoxins	Corn	Driehuis (2013)
Aspergillus, Penicillium and *Fusarium*	Aflatoxins	Corn silage	Alonso *et al*. (2013)
Alternaria	Tenuazonic acid	Cereals	De Oliveira *et al*. (2014)
Aspergillus and *Penicillium*	Aflatoxins, zearalenone, deoxynivalenol and fumonisin	Corn silage	Alonso *et al*. (2013)
Fusarium	Deoxynivalenol and nivalenol	Corn silage, rye and barley	Driehuis (2013)
Claviceps	Ergot	Sorghum	De Oliveira *et al*. (2014)
Aspergillus, *Fusarium* and *Penicillium*	Aflatoxins, ochratoxin A, deoxynivalenol, fumonisins and T-2	Corn silage	Biro *et al*. (2009)

fibrous and generally represent the vegetative portion of the plants. The digestible energy content of forages usually comes from cellulose or hemicellulose. Complete rations or Total Mixed Rations (TMR), are defined as those with all the forage and concentrate ingredients blended together, formulated to specific nutrient concentration and fed free-choice. The TMR has a potential higher risk of contamination by toxigenic fungi and mycotoxin, especially during storage. However, the main advantages of TMR feeding are that animals consume the desired proportion of forages when two or more forages are offered and the desired amount of forage relative to the amount of grain offered, there is a lower risk of digestive upsets; feed efficiency is improved, it also enables greater use of unpalatable and commodity feeds, greater accuracy in formulating and feeding and reduces labor in feeding.

TOXIGENIC FUNGI AND MYCOTOXIN CONTAMINATION IN SILAGE FOR DAIRY CATTLE

In intensive and semi-intensive dairy systems, animals are fed forage processed as hay, silage or green chop, all important strategies for feed storage in times of scarcity due to climate changes. These techniques are also important to reduce feed particles and make mixing with concentrate easier, preventing the selection of a given feed stuff when animals eat. Besides the seasonality of the forages, another negative factor related to animal productivity is the tendency for fungi development in feed products as a consequence of high temperature and humidity. To prevent fungi development, there should be measures in place for careful harvesting, transportation, storage and animal feeding processes. Good corn silage contains nearly 50% grain on a Dry Matter (DM) basis. It is an excellent source of energy for dairy cattle. If properly produced, animals will eat large amounts of this ingredient. Corn silage requires protein and mineral supplementation to be balanced for high milk production. To attain maximum yield, corn should be harvested for silage when it reaches physiological maturity, with dry matter content of about 35%. In the silo, leakage losses occur when material with less than 32% DM is ensiled. If corn becomes too dry before ensiling, losses are greater and the feed may be poorly compacted and present higher mold contamination.

Molds can develop on various types of animal feedstuffs at various growth stages, before harvest, or during storage. When weather conditions are right for their growth, molds can develop on grain kernels still attached to plants in the field and produce mycotoxins. Some grains (corn and wheat) may show significant levels of mycotoxin at harvest. Molds can also proliferate on stored feed, such as corn silage, if oxygen leaks into the silage mass. Moldy feeds usually decrease dairy performance parameter by 10%, even if they do not produce mycotoxins, as cows may find moldy feeds less palatable and therefore decrease their dry matter intake. Hence, lower nutrient intake reduces milk yield and body weight gain. When mycotoxins contaminate feed, they can cause

Table 2: Toxic effects of the most important mycotoxins in cattle

Mycotoxins	Toxic effects	Reference
Aflatoxin	Affects immunological functions and rumen metabolism	Rossi *et al*. (2009)
	Decreases feeding efficiency in dairy cattle	Whitlow *et al*. (2010)
	Reduces milk yield	
	Causes damage to the liver	Abidin and Khatoon (2012)
	Decreases feeding efficiency in dairy cattle	
	Hepatotoxic, Immunosupressive	Cigic and Prosen (2009)
Ochratoxin	Causes kidney problems	Cigic and Prosen (2009)
	Reduces milk yield	
Fumonisin	Causes reproductive problems	
	Negatively affects the function of the immunological system	Voss *et al*. (2011)
	Causes lesions in kidneys and liver	Upadhaya *et al*. (2010)
Zearalenone	Causes infertility	
	Reduces milk yield	
Deoxynivalenol	Causes hyperestrogenism	
	Negatively affects the function of the immunological system	Upadhaya *et al*. (2010)
	Gastrointestinal effects	Sultana and Hanif (2009)
	Gastrointestinal effects	Rossi *et al*. (2009)
T-2	Acute hemorrhagic enteritis	
	Reduces feed intake	

problems even at extremely low levels of ingestion. High-producing cows are highly susceptible to mycotoxins and animals that are already stressed by other health issues, such as lameness, high temperature or improper feed are at increased risk. Mycotoxin contamination can come from purchased feed sources, farm fields or silos. The fermentation process in silage does not break down mycotoxins in contaminated feeds and in some cases, may lead to the concentration of toxins in the grains (corndistiller's grains). Harmful levels of mycotoxins might be involved in low performance, acute clinical symptoms or both (Whitlow *et al*., 2010).

The most important mycotoxins found in silage of different countries involve those produced by fungi in the genera *Aspergillus* (aflatoxins B_1, B_2, G_1 and G_2) and *Fusarium* (deoxynivalenol-DON or vomitoxin-zearalenone, T-2 toxin and fumonisins B_1 and B_2). These mycotoxins may cause different problems to the animals and may lead to death in more serious cases (Dell'Orto *et al*., 2015; De Oliveira *et al*., 2014). In a study carried out in the Netherlands, Van Asselt *et al*. (2012) reported a high incidence of *Fusarium* species (50% of the corn samples that were analyzed were contaminated) and their mycotoxins as contaminants of corn silage. In the total of samples analyzed, 11 different *Fusarium* species were identified. The most common ones were *F. crookwellense* or *cerealis*, followed by *F. graminearum*, *F. culmorum*, *F. equiseti* and *F. sporotrichioides*. In Brazil, a study describing the occurrence of mycotoxins in silage offered to cattle reported the incidence of aflatoxin B_1 in 3 samples of corn silage out of 36 samples analyzed, at mean level of 13.02 $\mu g\ kg^{-1}$ (Bernardes *et al*., 2012).

TOXIC EFFECTS OF MYCOTOXINS IN DAIRY COWS

Ruminants are less susceptible to the effects of mycotoxins, when compared with monogastric animals. This difference may be partially attributable to the presence of the rumen, once this organ is populated by microorganisms (fungi, bacteria, protozoa, archaea). Among these, protozoans are the major microorganisms responsible for mycotoxin detoxification. This process, however, is not always efficient: In certain conditions, microorganisms produce an opposite effect, increasing the toxic action of the mycotoxin, such as when zearalenone is transformed into α-zearalenol, a compound four times more toxic than the parent compound (Dell'Orto *et al*., 2015). The main effects of mycotoxins (aflatoxin, ochratoxin, fumonisin, zearalenone, deoxynivalenol and toxin T-2) are described in Table 2.

In early stages of intoxication, mycotoxins cause relatively minor problems to dairy cows. The decline in performance may be negligible. Within days or weeks, the effect of continued mycotoxin consumption on performance (milk production or body weight gain) becomes more evident. Unspecific signs including loss of appetite, ketosis and displaced abomasum may be significantly increased by the ingestion of mycotoxins. Some mycotoxins can determine toxic effects in target organs, such as zearalenone, which causes estrogenic effects, swollen vulvas and nipples and vaginal prolapses. Reduced conception rates or abortion may also be related to mycotoxin consumption. The effects of mycotoxins are amplified by production stress: high-producing dairy cows are more susceptible to the effects of mycotoxins than low-producing ones (Abidin and Khatoon, 2012).

Acute aflatoxicosis is characterized by quick deterioration of general status, loss of appetite, low feed conversion, interference with reproductive capacity, immuno suppression, acute hepatitis, jaundice, hemorrhage and death (Abidin and Khatoon, 2012; Whitlow *et al*., 2010). The liver is the most importantly affected organ, with lesions caused by hemorrhagic necrosis, centrolobular congestion, proliferation of cells of biliary ducts and fatty infiltration in hepatocytes (De Oliveira *et al*., 2014). The effects of chronic toxicity are also characterized by hepatic lesions in a lower extent and include changes in growth of rumen microorganisms and genetic changes (Whitlow *et al*., 2010).

The main clinical manifestations of aflatoxicosis in dairy cows include reduced feed intake and milk yield, weight loss, lameness and jaundice. The liver exhibits signs of fatty degeneration, necrosis, increased volume and pale color, fibrosis and carcinomas. There are also signs of kidney degeneration related to renal excretion of metabolites from liver detoxification of the toxins (Abidin and Khatoon, 2012; Whitlow *et al*., 2010). Aflatoxicosis in cattle may be classified in three distinct clinical forms: (a) Primary acute disease, showing a variable picture as a function of sensitivity, generally after the intake of moderate to high amounts of AFB_1, with lesions in liver, kidneys and central nervous system, (b) Primary chronic disease, after the intake of moderate to low amounts of AFB_1 that are unable to cause classical clinical picture, although there is a negative effect on health and reproduction, leading to delayed growth, loss in carcass quality and reduced milk yield (Whitlow *et al*., 2010) and (c) Secondary chronic disease caused by the intake of small amounts of aflatoxin that are unable to cause evident clinical intoxication, but are able to predispose the animal to secondary diseases by immunosuppression (Upadhaya *et al*., 2010; De Oliveira *et al*., 2014).

Besides the important impact on the health and performance of dairy cows, the intake of feed contaminated with aflatoxin B_1leads to its biotransformation into aflatoxin M_1 (AFM_1) by liver enzymes of cytochrome P450. This hydroxylated derivative is water soluble and may be easily excreted in body fluids, such as urine and milk, which represents a serious risk to human health. Economic losses caused to milk producers due to the chronic toxic effects of aflatoxins may be less obvious than those caused by acute intoxications. While losses caused by mortality, decreased yield and milk contamination may be estimated, other less evident effects, such as infertility, reduced rumen and feed efficiency, immunological problems and other performance losses may be more difficult to be estimated.

RESIDUES OF MYCOTOXINS IN MILK

The AFM_1 is detected in the milk few hours after the intake of the contaminated feeds tuff, disappearing from the milk two to three days after the feed stuff is removed from the diet (De Oliveira and Corassin, 2014). Therefore, the presence of AFM_1 residues in milk and dairy products is a public health concern, taking into account their potential for additional aflatoxin exposure of humans through the diet. For this reason, several countries established maximum levels for AFM_1 in milk and milk products, although, other mycotoxins may also be excreted into milk. All over the world and especially in the European Union, there are also strict regulations for the maximum tolerable concentration of aflatoxin B_1 in feed ingredients and rations for dairy cows (De Oliveira *et al*., 2014).

The presence of AFM_1 in milk and dairy products is a worldwide concern, once these products are frequently consumed and milk is the one of the most important foods for children (De Oliveira *et al*., 2014). Evidence of human exposure to AFM_1 due to the consumption of milk and dairy products has been well demonstrated worldwide. In Iran, Ghazani (2009) observed that all 50 samples of milk analyzed (n = 50) were contaminated with AFM_1, with 62% of samples at levels above 0.05 $\mu g\ L^{-1}$, which is limit adopted by the European community. In another survey conducted in Iran, Heshmati and Milani (2010) found 33% of samples of UHT milk analyzed with AFM_1 levels higher than 0,05 $\mu g\ L^{-1}$. Bilandzic *et al*. (2010) found 98% of 61 samples of raw milk traded in Croatia contaminated with concentrations up to 0.5 $\mu g\ L^{-1}$. In Syria, Ghanem and Orfi (2009) found mean concentrations of 0.492 $\mu g\ L^{-1}$ in 126 samples, from which 80% showed levels above the maximum limit determined by the European Community. Studies conducted in Brazil also indicate high incidences of AFM_1 in milk and dairy products, although at relatively low levels (below 0.05 $\mu g\ L^{-1}$) (De Oliveira *et al*., 2010).

PREVENTION AND DECONTAMINATION STRATEGIES FOR MYCOTOXINS IN THE DIET FOR DAIRY COWS

Concerns with the negative impacts of mycotoxins on health and economy led to investigations for strategies to prevent toxin production in foodstuffs, as well as to eliminate, inactivate or reduce their bio availability in contaminated products (Goncalves *et al*., 2015). Contamination may be prevented by good agricultural practices, antifungal agents, genetic engineering and storage control. Mycotoxin elimination or inactivation may be achieved by physical, chemical and biological methods (Corassin *et al*., 2013). Entero-adsorption is used to reduce bioavailability by the addition of adsorbent compounds that are nutritionally inert to the diet. These compounds are able to sequester mycotoxins and prevent their absorption in the gastrointestinal tract of the animals (De Oliveira and Corassin, 2014).

The main physical methods used for mycotoxin decontamination are thermal inactivation, ultraviolet light, ionizing radiation, or extraction with solvents. Chemical methods include chlorination and oxidant or hydrolytic agents. However, both chemical and physical methods have advantages and disadvantages, as they do not remove the toxin completely, are expensive and cause nutritional and organoleptic losses to the products (De Oliveira *et al*., 2014). Biological methods are based on the action of microorganisms such as yeasts, molds, bacteria and algae on mycotoxins, by means of competition with nutrients and space, interaction and antibiosis (Fazeli *et al*., 2009). The use of microorganisms is an attractive alternative for the control and elimination of aflatoxins in foodstuffs and animal feed, protecting their quality and safety. *Saccharomyces cerevisiae* is the most well-known and commercially important yeast and its strains are widely used in the production of alcoholic drinks and in the bread industry. *In vitro* studies have demonstrated the ability of *S. cerevisiae* to remove AFB_1 from the substrate, although not all strains of a given genus and/or species have equivalent capacity of removing the toxin (Corassin *et al*., 2013). In fact, this ability is a characteristic of specific lineages, with highly variable efficacy (Goncalves *et al*., 2015).

Some physical, chemical and enzymatic treatments may increase the ability of *S. cerevisiae* to bind to aflatoxin in the substrate. Rahaie *et al*. (2010) observed that heat treatment may increase the permeability of the external layer of the cell wall due to the suspension of some mannanes on the cell surface, leading to increased availability of previously hidden binding sites. Corassin *et al*. (2013) used *S. cerevisiae* inactivated by heat, either alone or in combination with lactic acid bacteria and observed high efficiency (>90%) in AFM_1 reduction in UHT milk treated with *S. cerevisiae* for a relatively short period (30 and 60 min).

Besides cell viability, other factors may influence the ability of *S. cerevisiae* to remove aflatoxins, such as bacterial concentration, specificity of the bacteria, incubation temperature, pH and addition of nutrients, among others. It should be emphasized that the stability of the *S. cerevisiae* aflatoxin complex is a critical factor to prevent the release of the toxin during the passage through the gastrointestinal tract, as the complex has to resist stressors such as low pH and presence of bile. However, *in vitro* tests are not always reliable indicators of the adsorption of aflatoxin by *S. cerevisiae* during this passage. Therefore, *in vivo* evaluations are necessary to determine the efficacy of these microorganisms in binding to aflatoxinin animal feed and their ability to keep the toxin adsorbed, similar to what is recommended for mineral adsorbents used for mycotoxins.

CONCLUSION

Mycotoxins are frequently found in feed and silage used for dairy cattle. Zearalenone, aflatoxins and DON are the most common ones, leading to large and worldwide economic losses. Feeds contaminated with aflatoxin B_1 leads to the excretion of AFM_1 in milk of dairy cows. Good agricultural, storage and management practices should be a priority to prevent the presence of undesirable fungi in feed ingredients and the consequent mycotoxin production. Mycotoxin-contaminated feed may be submitted to physical, chemical or biological methods to reduce or eliminate some toxins depending on the type and concentration of mycotoxin, among other factors.

Dairy cows are constantly exposed to mycotoxins in feed preserved as hay and silage. The effects of these toxins in dairy cattle may vary according to the mycotoxin and include reduced feed intake, decreased milk yield, gastrointestinal disorders, immunosuppression and increased somatic cell counts in milk, lower reproductive efficiency, signs of fatty degeneration, necrosis and carcinomas in the liver. Further studies on toxicological effects of mycotoxins in dairy cows are necessary for a complete risk evaluation aiming to establish regulations for feedstuffs intended for dairy cattle. Routine analyses of mycotoxin should be implemented in dairy farms that use silage.

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