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# *Silage review:* Mycotoxins in silage: Occurrence, effects, prevention, and mitigation[1]

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## ABSTRACT

Ensiled forage, particularly corn silage, is an important component of dairy cow diets worldwide. Forages can be contaminated with several mycotoxins in the field pre-harvest, during storage, or after ensiling during feed-out. Exposure to dietary mycotoxins adversely affects the performance and health of livestock and can compromise human health. Several studies and surveys indicate that ruminants are often exposed to mycotoxins such as aflatoxins, trichothecenes, ochratoxin A, fumonisins, zearalenone, and many other fungal secondary metabolites, via the silage they ingest. Problems associated with mycotoxins in silage can be minimized by preventing fungal growth before and after ensiling. Proper silage management is essential to reduce mycotoxin contamination of dairy cow feeds, and certain mold-inhibiting chemical additives or microbial inoculants can also reduce the contamination levels. Several sequestering agents also can be added to diets to reduce mycotoxin levels, but their efficacy varies with the type and level of mycotoxin contamination. This article gives an overview of the types, prevalence, and levels of mycotoxin contamination in ensiled forages in different countries, and describes their adverse effects on health of ruminants, and effective prevention and mitigation strategies for dairy cow diets. Future research priorities discussed include research efforts to develop silage additives or rumen microbial innocula that degrade mycotoxins.
**Key words:** animal performance, health, mycotoxin

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## INTRODUCTION

Mycotoxins are a group of secondary metabolites secreted by fungal organisms mostly belonging to the genera *Aspergillus*, *Fusarium*, *Alternaria*, and *Penicillium* (Yiannikouris and Jouany, 2002; Bennett and Klich, 2003; Kabak et al., 2006). Several fungal species belonging to these genera can produce mycotoxins such as aflatoxins, ochratoxins, trichothecenes, zearalenone (**ZEA**), fumonisins, and several other mycotoxins considered minor or emerging mycotoxins (Bennett and Klich, 2003; Gallo et al., 2015, 2016; Loi et al., 2017). Fungi often thrive well in environments with high humidity, high temperature, and oxygen access during all stages of plant production and storage (Egal et al., 2005). Toxic effects, such as reduced feed intake and milk production, reproductive problems, immunosuppression, and death can occur when animals are fed mycotoxin-contaminated diets (Whitlow and Hagler, 2005; Zain, 2011).

Mycotoxins are present in a range of livestock feeds including concentrates, green forages, hays, and silages (Biomin, 2016). A 3-yr (2009 to 2011) survey on the worldwide occurrence of mycotoxins revealed that 81% of 7,049 livestock feed samples collected from the Americas, Europe, and Asia were positive for at least one mycotoxin (Rodrigues and Naehrer, 2012). Another recent 4-yr mycotoxin survey in Poland revealed that up to 95% of feedstuffs contained at least one mycotoxin (Kosicki et al., 2016). Most research studies have focused primarily on mycotoxin occurrence in cereal grains (Binder, 2007; Zinedine et al., 2007; Bhat et al., 2010), perhaps because they are consumed in greater quantities by humans than other feeds. Relatively few studies have surveyed mycotoxins in silages (O'Brien et al., 2006), which account for a major portion of dairy cow diets (Cheli et al., 2013). Yet a survey by Driehuis et al. (2008a) revealed that the contribution of ensiled forage to mycotoxin ingestion in dairy cows on 24 farms in the Netherlands was approximately 3 times more than that of other feed ingredients. A review of the literature reveals that the contribution of silage mycotoxins to the total amount of mycotoxins ingested by cows can be greater than the maximum concentrations allowed or recommended in ruminant diets by the US FDA and European Union (Table 1).

The ubiquitous nature of mycotoxins and the severity of the effects of some mycotoxins on human health make them a major food safety concern (CAST, 2003). In addition to the severity of their effects on livestock and human health, direct costs of disposal of condemned food and feed ingredients, and indirect costs of regulatory enforcement and quality control measures caused by fungal toxin contamination in the United States were estimated at approximately $1 billion per annum (CAST, 2003; Milicevic et al., 2010). The occurrence of mycotoxins in silage was recently reviewed by Wambacq et al. (2016), with particular emphasis on incidence and prevalence in Europe. However, our review gives more insight into the current silage mycotoxin prevalence surveys and studies around the world, including the consequent effects of the major mycotoxins on performance and health of ruminants. Therefore, the aim of this article is to review the literature on mycotoxin contamination of ensiled forages, including predisposing factors for mycotoxin production, to discuss the current state of knowledge on sequestration and degradation of mycotoxins in dairy cow diets, and to suggest future mycotoxin research priorities.

## FACTORS THAT PREDISPOSE TO MOLD AND MYCOTOXIN PRODUCTION IN SILAGE

Ensiled forages may contain a mixture of mycotoxins, originating from pre-harvest contamination by *Fusarium* and *Aspergillus* species (Baath et al., 1990; Uegaki et al., 2013; Gallo et al., 2015) and or from postharvest contamination with toxigenic molds that are common in silage, such as *Aspergillus* and *Penicillium* (Garon et al., 2006; Fink-Gremmels, 2008; Alonso et al., 2013). However, many common molds do not produce mycotoxins, and the presence of molds in silage does not indicate the presence of mycotoxins nor does their absence confirm mycotoxins are absent (Zain, 2011).

Both environmental and physiological conditions are known to influence synthesis of mycotoxins. Temperature, water activity (**aw**), and insect activity are the major factors influencing mycotoxin contamination of feedstuffs (Coulombe, 1993). Molds can grow between 10 and 40°C, between pH 4 and 8, and when aw is greater than 0.7 (Whitlow and Hagler, 2005; Magan and Aldred, 2007). However, the conditions for mold growth and for mycotoxin formation are not necessarily the same (Whitlow and Hagler, 2005). For instance, *Fusarium* molds can grow aggressively at 25 to 30°C without producing mycotoxins, whereas at freezing temperatures, they produce large quantities of mycotoxins with minimal growth (Joffe, 1986; Whitlow and Hagler, 2005).

Oxidative stress also often induces toxigenic pathways in various fungi (Reverberi et al., 2010; Ponts, 2015). This oxidative response, often defined by production of peroxide, is triggered by the host plant upon fungal infection and could promote synthesis of mycotoxins by fungi (Baidya et al., 2014; Ponts, 2015). The production of peroxide causes the biosynthesis of different mycotoxins by fungi (Sheridan et al., 2015). In support, a glycogen synthase kinase gene in *Fusarium*, with putative role as a transcription factor that directly promotes mycotoxin production, was expressed following growth of the fungus under oxidative stress (Qin et al., 2015). An acidic pH (pH = 3) has also been suggested to be a prerequisite for deoxynivalenol (**DON**) production by *Fusarium* (Merhej et al., 2010). However, this does not agree with evidence of DON production on the field or during storage because pH is rarely as low as 3.0 in growing crops or stored feeds. In addition, DON production may not be attributed to acidic pH values because oxidative stress and acidification often occur together in fungi (Pérez-Sampietro and Herrero, 2014).

Molds can grow in wet feeds such as silage, if oxygen is not limiting (Whitlow and Hagler, 2005). Delayed harvesting, slow or delayed filing of silos, inadequate packing and sealing of silos, slow feedout rates, bridging in silage bags, and damaged plastic wrap, bags, or silo covers can create a conducive microclimate for mold proliferation and mycotoxin production (Whitlow and Hagler, 2005). Other factors that may predispose feeds to mold growth and mycotoxin production include physical damage to corn ears (Teller et al., 2012) and damage to plants and silo covers by rodent, rain, hail, and drought.

## TYPES OF MYCOTOXINS IN ENSILED FORAGES

More than 400 mycotoxins occur naturally; however, only a few have been studied extensively (Njumbe et al., 2015; Fromme et al., 2016). Mycotoxins that are frequently present in ensiled forages include trichothecenes, fumonisins, aflatoxins, ZEA, mycophenolic acid, and roquefortine C (Driehuis et al., 2008b; Schmidt et al., 2015).

**Table 1**. Estimated dietary contributions of whole-plant corn silage to total mycotoxin intake[1] in dairy cow diets

| Reference | Country | Mycotoxin | Mean concentration in corn silage (µg/kg of DM) | Maximum concentration in corn silage (µg/kg of DM) | Mean contribution of corn silage mycotoxin (% of total daily intake of mycotoxin in dairy diet DM)[2] | Maximum contribution of corn silage mycotoxin (% of total daily intake of mycotoxin in dairy diet DM)[3] |
|---|---|---|---|---|---|---|
| Richard et al., 2009 | France | $AFB_1$ | 28.0 | 51.3 | 42 (168)[4] | 77.0 (308) |
| Keller et al., 2013 | Brazil | $AFB_1$ | 33.0 | 54.0 | 49.5 (198) | 81.0 (324) |
| Schmidt et al., 2015 | Brazil | $AFB_1$ | 3.00 | 6.00 | 4.50 (18.0) | 9.00 (36.0) |
| Driehuis et al., 2008b | the Netherlands | ZEA | 174 | 943 | 13.1 | 70.7 |
| Storm et al., 2014 | Denmark | ZEA | 66.0 | 311 | 3.96 | 23.3 |
| Schollenberger et al., 2006 | Germany | ZEA | 432 | 1,790 | 25.9 | 134 |
| Eckard et al., 2011 | Switzerland | ZEA | 181 | 430 | 10.8 | 32.3 |
| Schmidt et al., 2015 | Brazil | ZEA | 181 | 1,830 | 10.8 | 137.3 |
| Kosicki et al., 2016 | Poland | ZEA | 97.8 | 696 | 5.8 | 41.7 |
| Storm et al., 2010 | Denmark | DON | 1,056 | 5,094 | 6.34 | 38.2 |
| Driehuis et al., 2008b | the Netherlands | DON | 854 | 3,142 | 5.12 | 23.6 |
| Storm et al., 2014 | Denmark | DON | 1,629 | 2,974 | 9.77 | 22.3 |
| Gallo et al., 2016 | Italy | DON | 280 | 560 | 1.68 | 4.20 |
| Schollenberger et al., 2006 | Germany | DON | 2,919 | 3,944 | 17.51 | 29.6 |
| Eckard et al., 2011 | Switzerland | DON | 1,356 | 2,990 | 8.14 | 22.4 |
| Keller et al., 2013 | Brazil | DON | 1,610 | 3,420 | 9.66 | 25.7 |
| Mansfield et al., 2005 | United States | DON | 1,400 | 5,100 | 8.40 | 38.3 |
| Kosicki et al., 2016 | Poland | DON | 1,222 | 7,860 | 7.33 | 47.2 |
| Driehuis et al., 2008b | the Netherlands | Fumonisin $B_1 + B_2$ | NA[5] | 34,000 | NA | 68 (20.4) |
| Kim et al., 2004 | United States | Fumonisin $B_1$ | 615 | 1,824 | 1.23 (0.37) | 3.65 (1.09) |
| Mansfield et al., 2007 | United States | Fumonisin $B_1 + B_2$ | 3,000 | 30,400 | 6.00 (1.8) | 60.8 (18.2) |
| Schmidt et al., 2015 | Brazil | Fumonisin $B_1$ | 369 | 2,310 | 0.74 | 4.62 |
| Kosicki et al., 2016 | Poland | Fumonisins | 11.3 | 108 | | |
| Schneweis et al., 2000 | Germany | Mycophenolic acid | 690 | 23,000 | 0.69 | 23.0 |
| Storm et al., 2014 | Denmark | Mycophenolic acid | 9.00 | 52.0 | 0.01 | 0.05 |
| Gallo et al., 2016 | Italy | Mycophenolic acid | 1,760 | 48,000 | 1.76 | 48.0 |
| Auerbach et al., 1998 | Germany | Roquefortine C | 17,000 | 36,000 | 127.5 | 270 |
| Auerbach et al., 1998 | Germany | Roquefortine C | 5,470 | 28,000 | 41.025 | 210 |
| Mansfield et al., 2008 | United States | Roquefortine C | 380 | 5,710 | 2.85 | 42.8 |
| Storm et al., 2014 | Denmark | Roquefortine C | 173 | 189 | 1.30 | 1.42 |
| Gallo et al., 2016 | Italy | Roquefortine C | 740 | 32,000 | 5.55 | 240 |

[1]Estimated daily DMI of 25 kg; estimated daily corn silage intake of 7.5 kg of DM; estimated concentrations of mycotoxins in total diet DM: aflatoxin $B_1$ ($AFB_1$; 20 µg/kg, FDA, 2010; 5 µg/kg, European Commission, 2006b), deoxynivalenol (DON; 5 mg/kg, FDA, 2011, and European Commission, 2006b), total fumonisins (15 mg/kg, FDA, 2011; 50 mg/kg, European Commission, 2006b), zearalenone (ZEA; 0.5 mg/kg, European Commission, 2006), mycophenolic acid (300 mg/kg, Mohr et al., 2007), and roquefortine C (4 mg/kg; Haggblom, 1990).

[2]Mean percentage contribution of whole-plant corn silage to total dietary mycotoxin allowed or stipulated by US Food and Drug Administration (FDA) or European Union, calculated as [(mean concentration of mycotoxin detected in corn silage × estimated daily corn silage intake)/(maximum permissible mycotoxin in diet allowed or stipulated by US FDA or European Union × total diet DMI)] × 100.

[3]Maximum percentage contribution of whole-plant corn silage to total dietary mycotoxin allowed or stipulated by US FDA or European Union, calculated as [(maximum concentration of mycotoxin detected in corn silage × estimated daily corn silage intake)/(maximum permissible mycotoxin in diet allowed or stipulated by US FDA or European Union × total diet DMI)] × 100.

[4]Values in parentheses are estimates based on maximum permissible level of the mycotoxin in Europe.

[5]NA = not given.

## *FUSARIUM* TOXINS

*Fusarium* species are identified as field fungi because they proliferate during plant growth and maturation (Christensen et al., 1977; Bennett and Klich, 2003; Storm et al., 2008). Their growth is favored at high humidity (>70%) and temperatures that fluctuate between those on hot days and cool nights (CAST, 2003). Ensiling conditions are not favorable for *Fusarium* species, as they do not tolerate low pH and anaerobic conditions. Nevertheless, some *Fusarium* toxins are relatively stable or are not completely destroyed during ensiling (Binder, 2007; Boudra and Morgavi, 2008; Cheli et al., 2013; Uegaki et al., 2013). Hence, concentrations of *Fusarium* toxins detected in silage may reflect the contamination levels at the time of harvesting (Driehuis et al., 2008b).

### *Trichothecenes*

Trichothecenes consist of sesquiterpenoid metabolites produced by many fungal genera such as *Fusarium*, *Myrothecium*, *Phomopsis*, *Stachybotrys*, *Trichoderma*, and *Trichothecium* (Bennett and Klich, 2003). Trichothecenes are classified as either macrocylic, characterized by the presence of an ester-ether bridge between carbon-4 and carbon-15, or nonmacrocyclic, characterized by the absence of the ester-ether bridge (Chu, 1998; Bennett and Klich, 2003). The nonmacrocyclic trichothecenes are produced primarily by *Fusarium* species. The nonmacrocyclic trichothecenes are further classified into type A trichothecenes, characterized by a hydroxyl or acyl group at carbon-8 position, which include T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, scirpentriol, and diacetoxyscirpenol, and type B trichothecenes, characterized by a keto function at carbon-8 position, which include DON, nivalenol, acetylated DON, and fusarenon X (Chu, 1998; Bennett and Klich, 2003; Marin et al., 2013).

***T-2 and HT-2.*** These toxins are mainly produced by *Fusarium sporotrichioides* and *Fusarium poae*, though they can also be produced by some other *Fusarium* species (Edwards et al., 2009; Streit et al., 2012). They are more prevalent in cereal grains, such as oats and barley, than ensiled forages (Fels-Klerx, 2010; EFSA, 2011) and are produced under wet and warm climatic conditions (Xu et al., 2014). Approximately 1% of 127 corn silage samples collected from different countries contained T-2 toxin, and the maximum concentration was 14 μg/kg (Biomin, 2016).

Both toxins are extensively detoxified by ruminal microbiota (EFSA, 2011; Li et al., 2011). Nevertheless, T-2 is associated with gastroenteritis and intestinal hemorrhages (Hsu et al., 1972), immunosuppression (Gentry et al., 1984; Black et al., 1992), and reduced performance and reproductive problems in cattle (Kegl and Vanyi, 1991). Calves consuming 10,000 to 50,000 μg/kg of T-2 toxin experienced abomasal ulcers and sloughing of the ruminal papillae (Cheeke, 1998). Guidelines for safe levels in feed of T-2 or HT-2 toxins have not been established by the US Food and Drug Administration or by European legislative agencies; nevertheless, total diet concentrations close to or above the toxic dose (median lethal dose in rat = 10,000 μg/kg of BW) reported by Li et al. (2011) are not recommended.

***Deoxynivalenol.*** Deoxynivalenol is produced by *Fusarium graminearum*, *Fusarium nivale*, *Fusarium culmorum*, *F. poae*, *Fusarium roseum*, and *Fusarium tricinctum*. Cold, wet periods followed by a short, dry period favor their secretion (Diekman and Green, 1992). The toxin may occur when wet conditions coincide with warm days and cool nights (De Wolf et al., 2005).

A 3-yr survey of 7,049 livestock feed samples to estimate the worldwide occurrence of mycotoxins revealed that DON represents a frequent threat to livestock with a prevalence rate of 59% and an average contamination level of 1,104 μg/kg (Rodrigues and Naehrer, 2012). In another survey on the presence of mycotoxins in ensiled feeds in the Netherlands (Driehuis et al., 2008b), corn and wheat silages were found to be the major sources of DON in diets of dairy cattle, with average concentrations of 854 and 621 μg/kg, respectively, and maximum concentrations of 3,142 and 1,165 μg/kg, respectively. In another recent multiannual mycotoxin survey in Poland (Kosicki et al., 2016), DON was detected in 86% of the 143 corn silage samples at average and maximum concentrations of 223 and 7860 g/kg, respectively. A survey of corn, alfalfa, and sorghum silage samples from Argentinian dairy farms for mycotoxins was conducted from January 2012 to September 2015. The study focused on farms with suspected mycotoxin problems due to issues such as poor silo covering, low silage feed-out rates, low packing density, poor milk yield or quality, and poor reproductive performance. Analysis of the silage samples was performed by by Teklab Environmental Laboratory, Cordoba, Argentina. Approximately 28.2% of contaminated corn silages and 50.0% of contaminated alfalfa silages contained more than 2,000 μg/kg of DON (Table 4). These studies show that DON is one of the most commonly detected mycotoxin in silages and the concentrations can be very high (Storm et al., 2008; Gallo et al., 2015; Cogan et al., 2016).

Deoxynivalenol is also known as vomitoxin because it is associated with vomiting in swine, as well as feed refusal, diarrhea, reproductive problems, and eventual death (Canady et al., 2001). Ruminants are relatively resistant to DON (Pestka, 2007) because some rumen microorganisms can extensively convert DON to nontoxic, de-epoxy DON (Swanson et al., 1987; Marczuk et al., 2012). No reduction in milk production was observed when DON was fed to dairy cows at 66,000 or 6,400 μg/kg for 5 d or 6 wk, respectively (Cote et al., 1986; Trenholm et al., 1985). Similarly, Charmley et al. (1993) and Korosteleva et al. (2009) reported no reduction in milk production of dairy cows consuming a DON-contaminated diet (2.6 to 6.5 mg/kg). However, altered rumen fermentation and reduced protein flow to the duodenum (Danicke et al., 2005; Seeling et al., 2005; Table 2) were observed in dairy cows when 8.21 mg/kg of DM of DON and 0.09 mg/kg of ZEA were fed daily for 4 wk, possibly because of the synergistic toxic effects of ZEA and DON (Grenier and Oswald, 2011). The guidance level for DON in adult ruminant feeds in Europe is 5 mg/kg of DM (European Commission, 2006a; Table 3) and the advisory guidelines stipulated by the US FDA are 5 and 10 mg/kg of diet DM for dairy and beef cattle, respectively (FDA, 2011; Table 3).

Some other trichothecenes such as nivalenol, acetylated DON, diacetoxyscirpenol, and fusarenon X have also been isolated from silages (Schollenberger et al., 2006; Storm et al., 2010; Eckard et al., 2011). However, their presence in silages is sporadic, and mostly at low concentrations (Gallo et al., 2015; Wambacq et al., 2016).

### *Fumonisins*

Fumonisins are produced by several *Fusarium* species such as *Fusarium verticillioides*, *Fusarium proliferatum*, *Fusarium anthophilum*, and *Fusarium nygamai* (Bennett and Klich, 2003). They are synthesized by condensation of the AA alanine into an acetate-derived precursor (Bennett and Klich, 2003). More than 28 different forms of fumonisins are known, designated as A-series, B-series, C-series, and P-series (Yazar and Omurtag, 2008). Fumonisin B (and notably fumonisin $B_1$) are considered the most toxic with regard to feed contamination (Marasas, 1996; Schmaile and Munkvold, 2009). Fumonisin $B_1$ is mainly produced by *F. proliferatum* and *Fusarium verticillioides* (Marasas, 1996; Whitlow and Hagler, 2005), which are the 2 major causes of *Fusarium* ear rot, one of the most common diseases of corn plant (Gxasheka et al., 2015). Hot, dry periods followed by humid conditions and insect damage are the major predisposing factors that favor the secretion of fumonisins by *Fusarium* species (De Wolf et al., 2005). Fumonisins represent the most frequent threat to livestock among mycotoxins, with a 64% prevalence rate and an average concentration of 1,965 μg/kg for 7,049 livestock feed samples collected from the Americas, Europe, and Asia (Rodrigues and Naehrer, 2012). About 48.6% of 327 corn silages sampled in Brazil were contaminated with fumonisin $B_1$, with an aver-

**Table 2**. Summary of effects of common silage mycotoxins in ruminants[1]

| Mycotoxin | Summary of effects |
|---|---|
| Aflatoxins | Reduced milk production in dairy cows.<br>Decreased milk quality and safety due to carry-over of toxin from contaminated feed.<br>Decreased feed efficiency and rate of gain in beef cows.<br>Compromised immune and ruminal functions.<br>Liver malfunctions. |
| T-2 | Immunosuppression in cattle because of reduced antibody production, neutrophil function, and lymphocyte blastogenesis.<br>Bovine infertility and abortion in late gestation.<br>Extent of transfer into milk of ruminants is negligible. |
| Deoxynivalenol | Gastrointestinal problems and reduced performance probably due to feed refusal.<br>No evidence of carry-over into milk of ruminants. |
| Zearalenone | Infertility, reduced milk production, and hyperestrogenism in cattle.<br>Extent of transfer into milk of ruminants is negligible. |
| Fumonisins | Reduced performance probably due to feed refusal.<br>Mild liver disease.<br>Carry-over of the toxin into milk of ruminants is negligible. |
| Ochratoxins | No significant toxicity to cattle when fed alone in naturally occurring doses.<br>Carry-over of the toxin into milk is minimal. |
| Roquefortine | Feed refusal, reproductive disorders, and paralytic effects.<br>No evidence of transfer into milk of ruminants. |
| Mycophenolic acid | Research data on the effects in cattle are lacking.<br>No evidence of transfer into milk of ruminants. |

[1]Sources: Coppock et al., 1990; Black et al., 1992; Cheeke, 1998; Auerbach et al., 1998; Diaz et al., 2000; Mathur et al., 2001; Yiannikouris and Jouany, 2002; Danicke et al., 2005; Seeling et al., 2005; Battacone et al., 2005; Fink-Gremmels, 2008; Korosteleva et al., 2009; Zain, 2011; Queiroz et al., 2012; Ogunade et al., 2016; Hashimoto et al., 2016.

**Table 3**. Regulatory levels of common mycotoxins in ruminant feeds and human foods

| Common mycotoxin | Agency | Intended use: Ruminant feeding | Intended use: Human consumption |
|---|---|---|---|
| Aflatoxins | US Food and Drug Administration | 20 μg/kg for dairy animals<br>100 μg/kg for breeding cattle<br>300 μg/kg for finishing beef cattle | 20 μg/kg in all foods intended for human consumption<br>0.50 μg/kg in milk (aflatoxin $M_1$) |
| | European Commission | 5 μg/kg for dairy animals<br>20 μg/kg for all other ruminants | 4 μg/kg in all cereals and cereal products with the exception of corn and rice subjected to physical treatment (10 μg/kg)<br>0.1 μg/kg in foods for infants and young children<br>0.05 μg/kg in milk (aflatoxin $M_1$) |
| Deoxynivalenol | US Food and Drug Administration | 10 mg/kg for ruminating beef and feedlot cattle (>4 mo old)<br>5 mg/kg for ruminating dairy cattle older than 4 mo | 1 mg/kg in wheat products intended for human consumption |
| | European Commission | 5 mg/kg for ruminants | 0.75 mg/kg in cereals intended for direct human consumption<br>0.2 mg/kg in foods for infants and young children |
| Total fumonisins | US Food and Drug Administration | 15 mg/kg for breeding ruminants<br>30 mg/kg for ruminants at least 3 mo old raised for slaughter | 2 mg/kg in degermed dry milled corn products<br>3 mg/kg in popcorn<br>4 mg/kg in whole or partially degermed dry milled corn products and dry milled corn bran |
| | European Commission | 50 mg/kg for adult ruminants (>4 mo old) | 1 mg/kg in corn intended for direct human consumption<br>0.2 mg/kg in foods for infants and young children |
| Zearalenone | US Food and Drug Administration | Currently not stipulated | Currently not stipulated |
| | European Commission | 0.5 mg/kg for ruminants | 0.075 mg/kg in cereals intended for direct human consumption<br>0.02 mg/kg in foods for infants and young children |
| Ochratoxins | US Food and Drug Administration | Currently not stipulated | Currently not stipulated |
| | European Commission | Currently not stipulated | 0.003 mg/kg in cereals intended for direct human consumption<br>0.0005 mg/kg in foods for infants and young children |
| T-2 and HT-2 | US Food and Drug Administration | Currently not stipulated | Currently not stipulated |
| | European Commission | Currently not stipulated | 0.05 mg/kg in foods intended for direct human consumption with the exception of corn (0.1 mg/kg) and oats (0.2 mg/kg)<br>0.015 mg/kg in foods for infants and young children |

age concentration of 369 μg/kg (Schmidt et al., 2015). Gonzalez-Pereyra et al. (2008) reported that fumonisin levels in corn silage varied from 340 to 2,490 μg/kg of DM, with higher concentrations detected in samples taken from the top layer and side walls of the silo, which are normally prone to air infiltration and less extensive fermentation.

The structural formula of fumonisin is similar to sphingosine, which is a component of sphingolipids found at high concentrations in nerve tissues (Michaelson et al., 2016). Fumonisins interrupt sphingolipid biosynthesis (Diaz and Boermans, 1994), thereby causing leucoencephalomalacia, also known as moldy corn poisoning, in horses (Kellerman et al., 1990), pulmonary edema in swine (Colvin and Harrison, 1992), and hepatotoxicity in rats, mice, and rabbits (Gumprecht et al., 1995; Voss et al., 1995). Because it is rumen degradable (60 to 90%), the toxin tends to be less potent in ruminants (WHO, 2000); however, fumonisin has been identified to be nephrotoxic to calves fed 1,000 μg/kg BW of

**Table 4**. Occurrence of deoxynivalenol in silage samples submitted for analysis from farms with suspected mycotoxin problems in Argentina

| Item | No. of samples: >2,000 μg/kg | No. of samples: <2,000 μg/kg | Concentration (μg/kg): Minimum | Maximum | Average | SD |
|---|---|---|---|---|---|---|
| Corn silage | 11 | 28 | 620 | 3,570 | 1,550 | 770 |
| Alfalfa silage | 6 | 6 | 900 | 4,840 | 2,150 | 1,200 |
| Sorghum silage | 0 | 2 | 250 | 1,080 | 670 | 590 |

**Table 5**. Occurrence of zearalenone in silage samples submitted for analysis from farms with suspected mycotoxin problems in Argentina

| Item | No. of samples | | Concentration (μg/kg) | | | |
|---|---|---|---|---|---|---|
| | >300 μg/kg | <300 μg/kg | Minimum | Maximum | Average | SD |
| Corn silage | 15 | 18 | 36.4 | 1,774.8 | 436.4 | 423.1 |
| Alfalfa silage | 5 | 6 | 43.9 | 1,599.3 | 533.8 | 551.9 |
| Sorghum silage | 3 | 2 | 116.6 | 595.3 | 368.4 | 198.7 |

the toxin (Mathur et al., 2001). Similar results were observed in beef calves supplemented with 148 mg/kg of total fumonisin in the diet for 31 d (Osweiler et al., 1993). Diaz et al. (2000) reported lower milk yield in Holstein and Jersey cows fed diets containing 100 mg/kg of fumonisin from 7 d before calving to 70 d after parturition. Nevertheless, carry-over of the toxin into milk is typically negligible (Scott et al., 1994; Table 2), and hence the toxin poses no serious food safety concern for humans. However, fumonisin $B_1$ is considered a possible carcinogen (in group 2B, IARC, 2002; Loi et al., 2017). The US FDA advisory guideline for fumonisins is 15 mg/kg of diet DM for lactating dairy cows, 30 mg/kg of diet DM for breeding ruminants, and 60 mg/kg of diet DM for calves over 3 mo that are intended for slaughter (FDA, 2011; Table 3). The advisory value in Europe is 50 mg/kg of diet DM for adult ruminants (European Commission, 2006a; Table 3).

### *Zearalenone*

Zearalenone, a macrocyclic β-resorcyclic acid lactone, is an estrogenic metabolite produced by several species of *Fusarium* such as *F. graminearum*, *F. roseum*, *F. culmorum*, and *Fusarium crookwellense* (Kuiper-Goodman et al., 1987; Saeger et al., 2003). *Fusarium graminearum* occurs naturally in high-moisture corn and it has also been detected in moldy hay and pelleted feeds (CAST, 2003). It also co-occurs with DON in grain crops, such as corn, barley, and sorghum, at very low concentrations (CAST, 2003; Kosicki et al., 2016). High-humidity conditions with alternating low (11–14°C) and moderate (27°C) temperatures favor its production (Vigier et al., 1997; De Wolf et al., 2005).

Several studies have reported the occurrence of ZEA in ensiled forages. Whitlow and Hagler (2005) reported an average ZEA concentration of 525 μg/kg of DM and 30% incidence in 461 corn silage samples in the United States. Driehuis et al. (2008b) reported ZEA contamination in 13% of 120 grass silage and 50% of 140 corn silage samples in the Netherlands with average concentrations of 180 and 146 μg/kg, respectively. Reed and Moore (2009) detected up to 660 μg/kg and 79.8 mg/kg of ZEA in sorghum and alfalfa silages, respectively in a study in Australia. Zearalenone was the most common mycotoxin detected in a study that assessed the exposure of Danish cattle to mycotoxins from corn silage (Storm et al., 2014). A survey reported that approximately 45% of 7,049 livestock feed samples collected from the Americas, Europe, and Asia contained ZEA, with an average concentration of 233 μg/kg (Rodrigues and Naehrer, 2012). A survey of corn, alfalfa, and sorghum silage samples collected from dairy farms in Argentina with suspected mycotoxin problems revealed that 45.5% of contaminated corn silages, 45.5% of contaminated alfalfa silages, and 60% of contaminated sorghum silages contained more than 300 μg/kg of ZEA, with maximum concentrations of 1,774.8, 1,599.3, and 595.3 μg/kg, respectively (Table 5).

The structural similarity of ZEA to estrogen enables the toxin to mimic the hormone (Saeger et al., 2003). This causes numerous reproductive problems, including hyper-estrogenism, vaginitis, and mammary gland enlargement, particularly in swine (Diekman and Green, 1992; Marczuk et al., 2012). Ruminants are less susceptible than swine to the toxin because ruminal microbiota, notably protozoa, have the potential to convert ZEA to its hydroxyl-metabolites, α- and β-zearalenol (Kennedy et al., 1998). Although α-zearalenol has higher affinity for estrogen receptors than ZEA, its lower absorption rate and or interconversion to β-zearalenol in the liver decrease its negative effects (Malekinejad et al., 2006; Fink-Gremmels, 2008). Nevertheless, the detoxification capacity of ruminal microbes may be exceeded by high intakes of ZEA. Weaver et al. (1986) reported reduced conception rates in dairy heifers fed 12,500 μg/kg of ZEA. Furthermore, decreased intake and milk production, diarrhea, and reproductive failure were reported in heifers fed diets contaminated with 660 μg/kg of ZEA and 440 μg/kg of DON (Coppock et al., 1990).

The major concern with ZEA toxicity is its negative effect on animal health and reproduction because, like fumonisins, the extent of transfer into milk is negligible (Seeling et al., 2005; Table 2). Consequently, there are no FDA action limits, guidance, or advisory levels for ZEA. However, the guidance value for ruminant feeds in Europe is 500 μg/kg (European Commission, 2006a; Table 3).

**Table 6**. Occurrence of aflatoxins in silage samples submitted for analysis from farms with suspected mycotoxin problems in Argentina

| | No. of samples | | Concentration (μg/kg) | | | |
|---|---|---|---|---|---|---|
| Item | >20 μg/kg | <20 μg/kg | Minimum | Maximum | Average | SD |
| Corn silage | 13 | 30 | 4.00 | 42.1 | 15.82 | 9.44 |
| Alfalfa silage | 5 | 7 | 7.06 | 30.5 | 16.9 | 8.45 |
| Sorghum silage | 0 | 5 | 6.45 | 15.3 | 9.85 | 3.22 |

Other *Fusarium* mycotoxins that have been detected in silages include enniatins, beauvericins, fusaric acid, and moniliformin (Sørensen et al., 2008; Shimshoni et al., 2013; McElhinney et al., 2015); however, their occurrences are sporadic or rarely reported, and their toxicological effects in ruminants have not been properly characterized (Sulyok et al., 2010; Shimshoni et al., 2013).

## *ASPERGILLUS* TOXINS

*Aspergillus* molds are considered storage fungi because they usually do not infect crops before harvest. However, some species such as *Aspergillus flavus* may infect crops and produce aflatoxins in the field (Storm et al., 2008) during periods of high temperature (>32°C), high humidity (>80%), or during drought stress (De Wolf et al., 2005; Tsitsigiannis et al., 2012). Insect damage to forages can also predispose to or excarcebate the infection.

### *Aflatoxins*

Aflatoxins are a group of toxic, mutagenic, and carcinogenic difuranocoumarin derivatives produced by a polyketide pathway by many strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nominus* (Deiner et al., 1987; Kurtzman et al., 1987; Bennett and Klich, 2003). Natural forms of aflatoxins such as $B_1$ and $G_1$, and their dihydroderivatives, $B_2$ and $G_2$, are naturally present in food and feed ingredients because of the ability of *Aspergillus* to grow over a broad range of temperatures and humidities (Dutton, 1988; Tulayakul et al., 2005). Nevertheless, *Aspergillus* spp. are prevalent primarily in regions with tropical or subtropical climates (Cheli et al., 2013). Aflatoxin $B_1$ (**$AFB_1$**) is the main toxin and the most potent natural carcinogen produced by toxigenic *Aspergillus* species (Squire, 1981). The incidence and concentration of aflatoxin are relatively low in well-preserved silages compared with other mycotoxins (Richard et al., 2009; Keller et al., 2013; Schmidt et al., 2015; Kosicki et al., 2016). Aflatoxins were present in 33% of 7,049 livestock feed samples at an average concentration of 63 μg/kg in the intercontinental survey of Rodrigues and Naehrer (2012). In a survey of Argentinian dairy farms with suspected mycotoxin problems (Table 6), approximately 30.2% of corn silage samples and 41.7% of alfalfa silage samples contained more than 20 μg/kg of aflatoxins, with maximum concentrations of 42.1 and 30.5 μg/kg, respectively. In another survey conducted in the Netherlands by Driehuis et al. (2008b), no $AFB_1$ was detected in 140 corn silage samples. Likewise, no aflatoxin was detected in 25 out of 26 corn silage samples surveyed in Poland by Kosicki et al. (2016). However, high concentrations of aflatoxin can occur in poorly made silages or in silages made with diseased corn plants (Gonzalez-Pereyra et al., 2008; Queiroz et al., 2012). In addition, aflatoxins are prevalent in trench silos (Gonzalez Pereyra et al., 2011) more so than in silo bags because trench silos are often made over a concrete base or on the bare ground, and are more exposed to environmental conditions (Alonso et al., 2013). Up to 51 μg/kg of DM of $AFB_1$ was detected in corn silage collected in a trench silo in France (Richard et al., 2009). Furthermore, Gonzalez-Pereyra et al. (2008) reported an $AFB_1$ concentration of 156 μg/kg of DM in corn silage stored in a trench-type silo without proper sealing in Argentina.

Chronic exposure to aflatoxins in diets of lactating cows caused adverse effects such as reduced health and performance of cows, impaired liver function, suppressed immune status and increased susceptibility to diseases despite vaccinations (Garrett et al., 1968; Diekman and Green, 1992). The symptoms associated with acute aflatoxin ingestion (aflatoxicosis) include inappetence, ataxia, rough hair coat, and enlarged liver (Guthrie and Bedell, 1979; Whitlow and Hagler, 2005). Precise diagnosis of aflatoxicosis is difficult because the symptoms are not specific to the condition (Coulombe, 1993). Increased liver weight and reduced performance were observed in beef cattle fed a diet containing 100 μg/kg of aflatoxin (Garrett et al., 1968). Furthermore, reduced feed efficiency due to compromised ruminal function was reported in steers fed 600 μg/kg (Diekman and Green, 1992). Queiroz et al. (2012) noted decreased milk yield and milk protein concentration, as well as altered immune response in dairy cows fed 75 μg/kg of aflatoxin daily for 5 d. However, other studies reported no changes in milk yield when 170 (Kutz et al., 2009) or 112 μg/kg (Stroud, 2006) of $AFB_1$/kg of TMR DM were fed for 7 or 11 d, respectively. This contradiction may be because several factors, other than the amount ingested, may influence the response of cows to the toxin; such factors may include heat or acidotic stress, subclinical disease, and so on.

A portion of ingested $AFB_1$ can be degraded by ruminal microbes, resulting in the formation of aflatoxicol (Upadhaya et al., 2010). In humans and susceptible animals, the toxin is metabolized by cytochrome P-450 enzymes into aflatoxin-8, 9-epoxide, which is highly toxic, mutagenic, and carcinogenic, and which causes chromosome damage by binding with and forming adducts on DNA (Jouany et al., 2009). Other metabolic fates of $AFB_1$ in the liver include O-dealkylation to $AFP_1$, ketoreduction to aflatoxicol, and hydroxylation to aflatoxin $M_1$ (**$AFM_1$**; highly toxic and carcinogenic), $AFQ_1$, or $AFB_{2a}$ (Kuilman et al., 1998; Wu et al., 2009; Jafarian-Dehkordi and Pourradi, 2013). Aflatoxin $B_1$ is excreted in milk of dairy cows as $AFM_1$ (Diaz et al., 2004; Battacone et al., 2005). Contaminated milk and milk products represent a significant safety risk because, like $AFB_1$, $AFM_1$ is a group 1 human carcinogen (IARC, 2002). The rate of transmission of aflatoxin from feed into milk can be as low as 1% and as high as 6% (EFSA, 2004). Aflatoxin is the only mycotoxin that has government legislation for its maximum permissible level in feedstuffs and milk products. The Action Levels established by the US FDA are 0.5 and 20 μg/kg for liquid whole milk and feed ingredients offered to dairy cattle, respectively (FDA, 2011; Table 3), whereas the maximum levels recommended in Europe are 0.05 μg/kg for liquid whole milk and 5 μg/kg for feed ingredients (European Commission, 2006a; Table 3).

### Ochratoxins

Ochratoxins are secondary metabolites produced by *Penicillium verrucosum* or *Aspergillus ochraceus*, *Aspergilus niger*, and *Aspergillus carnonarius* (Frisvad et al., 2004; Frisvad and Samson, 2004; Keller et al., 2013). Ochratoxin A (**OTA**), which is carcinogenic and immunotoxic, and inhibits glucose metabolism, is a chloro-isocoumarin, and is considered the most toxic of this group of chemical compounds (Yiannikouris and Jouany, 2002).

The incidence and concentration of OTA in silage are usually low relative to other major mycotoxins such as DON, ZEA, fumonisin, and T-2 toxin (Biomin, 2016; Kosicki et al., 2016), probably because the fungi that produce this toxin cannot tolerate high concentrations of acetic acid and $CO_2$ (Frisvad et al., 2004; Frisvad and Samson, 2004; Gallo et al., 2015). For instance, 1% of 124 corn silage samples collected from different countries contained OTA and the maximum concentration was 3 μg/kg (Biomin, 2016). However, for unknown reasons, approximately 47% of 61 corn silage samples in Poland contained OTA, though the maximum concentration was only 7.5 μg/kg (Kosicki et al., 2016).

Ochratoxin A is extensively and rapidly degraded in the rumen to a less toxic product, ochratoxin-α (Hult et al., 1976; Fink-Gremmels, 2008), which explains tolerance of ruminants to the toxin. However, it also may be metabolized to ochratoxin C, which is similarly toxic (Chu, 1974). Bioavailability of dietary ochratoxin may be increased with high-grain diets, probably because they predispose to reduced ruminal pH (pH 5.5 to 5.8) (Hohler et al., 1999; Pantaya et al., 2016). Furthermore, the detoxifying capacity of the rumen may be exceeded at high feed intake, in which case the toxin can reduce milk production and be transferred into milk (Yiannikouris and Jouany, 2002).

## *PENICILLIUM* TOXINS

*Penicillium* molds can grow in typical silage environments with water activity levels (0.79–0.83 aw) that are relatively lower than those for other fungal organisms as well as under low oxygen concentration (1%) and low pH (3.0–6.0; Boysen et al., 2000; Nevarez et al., 2009). *Penicillium* molds are more common during storage, but they also can grow on plants under very wet conditions in the field (Christensen et al., 1977).

### PR Toxin, Mycophenolic Acid, and Roquefortine C

These are secondary metabolites of *Penicillium roqueforti* (PR) and *Penicillium paneum* (Schneweis et al., 2000; Nielsen et al., 2006). *Penicillium roqueforti* is commonly referred to as the silage mold (Pahlow et al., 2003) because it is acid-tolerant and it can grow at low oxygen concentrations (O'Brien et al., 2006). Consequently, it is the most frequently isolated *Penicillium* mold in silage (Sumarah et al., 2005). Up to 85% of 21 samples that had no visible mold silages surveyed in Germany contained *P. roqueforti* (Auerbach et al., 1998). Another survey of 20 corn silage stacks in Denmark revealed a 96% prevalence for *P. roqueforti* and *P. paneum* (Storm et al., 2010).

The PR toxin is rarely detected in ensiled forage (Gallo et al., 2016). However, mycophenolic acid and roquefortines are the most studied *Penicillium* toxins in ensiled forage (Gallo et al., 2015). In the Netherlands, roquefortine C was detected in 19% of 47 corn and grass silage samples and 7% of 29 samples of ensiled by-products with an average concentration of 780 μg/kg. Mycophenolic acid was detected in 13 and 10% of samples of these feed classes, respectively, with an average concentration of 0.52 mg/kg (Driehuis et al., 2008a). A recent study detected up to 7,600 μg of mycophenolic acid/kg in all 3 grass silage samples taken from areas with visible molds from different parts of bunker silos (Santos and Fink-Gremmels, 2014). Some other *Penicillium* toxins that have been isolated from ensiled forage include patulin, andrastin A, citreoisocumarin, agroclavine, festuclavine, and marcfortine (O'Brien et al., 2006; Storm et al., 2008, 2014).

Symptoms such as reproductive disorders, mastitis, and lack of appetite were detected in cattle herds fed silages containing 0.2 to 1.5 mg of roquefortine C/kg in northern Germany (Auerbach et al., 1998). Also, paralytic effects were reported in cows fed 4 to 8 mg of roquefortine C/kg (Haggblom, 1990). However, no clinical signs of intoxication were observed in sheep fed 25 mg of roquefortine C/kg for 18 d (Tuller et al., 1998), suggesting that sheep are more tolerant to roquefortine C than cattle. Mycophenolic acid is considered an immunosuppressive agent because it blocks the proliferative response of T and B lymphocytes, and inhibits antibody formation and production of cytotoxic T cells (Eugui et al., 1991). Research data on the effects of mycophenolic acid in cattle are lacking. However, Mohr et al. (2007) found no effects in sheep exposed to 10 to 300 mg of mycophenolic acid/kg daily for 44 d.

## *ALTERNARIA* TOXINS

*Alternaria* species such as *A. alternata*, *A. arborescens*, and *A. tenuissima* have been detected in ensiled feeds (Dacero et al., 1997; Mansfield et al., 2007; Ostry, 2008; Storm et al., 2008). Optimum growth of *A. alternata* occurs between 25 and 28°C, and at a water activity of approximately 0.88 aw (Hocking et al., 1994; Gravesen et al., 1994; Ostry, 2008). *Alternaria* species produce compounds such as alternariols, altertoxins, altenuene, tentoxin, phomapyrones, dehydrocurvularin, tenuazonic acid, pyrenochaetic acid, *Alternaria alternata* f. sp. lycopersici toxins, and several others (Andersen et al., 2002; Ostry, 2008). Alternariols have been reported to have DNA strand-breaking activities and several *Alternaria* toxins have been reported to be associated with human esophageal cancer in China (Liu et al., 1992; Pfeiffer et al., 2007; Storm et al., 2014). The ruminal degradability and toxic properties of these toxins have not been extensively studied and or confirmed in ruminants, and their occurrence has been sporadic and mostly at low concentrations in silage (Yu et al., 1999; Storm et al., 2014).

## CO-OCCURRENCE OF MYCOTOXINS IN LIVESTOCK FEEDS

Contamination of livestock feeds by multiple mycotoxins is a common occurrence on farms (Keller et al., 2013; Storm et al., 2014; Kosicki et al., 2016) because most fungi can produce several mycotoxins, and several fungi can simultaneously contaminate a single feed (Bottalico, 1998; Sweeney and Dobson, 1998; Alassane-Kpembi et al., 2017). Therefore, studying the occurrence and effects of single mycotoxins likely gives incomplete and or biased information about the associated risks. Scudamore et al. (1998) reported that all corn samples (n = 98) from 4 livestock feed mills in the United Kingdom contained more than one mycotoxin. In addition, almost half of 7,049 animal feed samples collected from the Americas, Asia, and Europe contained 2 or more of the following mycotoxins: DON, aflatoxins, ZEA, fumonisins, and OTA (Rodrigues and Naehrer, 2012). In a recent survey in Poland (Kosicki et al., 2016), the most frequently co-occurring mycotoxin combinations were DON and ZEA (81% of 143 corn silage samples). Aflatoxin $B_1$ was found to co-occur with OTA, ZEA, or with both toxins in 31, 12, or 27% of 123 Spanish barley samples, respectively (Ibáñez-Vea et al., 2012). In another study in the Netherlands, Driehuis et al. (2008a) reported that DON and ZEA co-occurred in 44% of dairy diets, and corn silage was the major source of both mycotoxins in the feeds. Furthermore, several studies have suggested that DON contamination can be considered as a potential marker of occurrence of other mycotoxins, especially the *Fusarium* toxins (Sobrová et al., 2010; Šliková et al., 2013).

Negative synergistic effects of several toxins in the diet can worsen health and production problems of cattle consuming mycotoxin-infested feeds. Santos and Fink-Gremmels (2014) reported high contamination levels of mycophenolic acid, DON, ZEA, and fumonisin $B_1$ in grass silages sampled from 3 farms in which dairy cows showed loss of body condition, signs of lameness with no clear disease, and low milk yield with increased SCC. In addition, a meta-analytic study that summarized 112 publications reported negative synergistic or additive effects of different mycotoxins on animal performance (Grenier and Oswald, 2011). This emphasizes the importance of studying co-occurrence of mycotoxins in feeds to get a clearer indication of their adverse effects on the performance and health of livestock.

## SILAGE MYCOTOXINS AND HUMAN HEALTH

Mycotoxins in silage can affect human health if animal-source foods (meat, milk, eggs, blood products, and so on) contaminated with these toxins are consumed. Human health effects of mycotoxins were reviewed by Wu et al. (2014a). Because the exposure route between humans and mycotoxins in silage is indirect (humans generally do not consume silage directly), no studies are available that confirm this particular exposure route and accompanying effects. However, ingested $AFB_1$ can be biotransformed by animals into its metabolite aflatoxin $M_1$, which also can have toxic effects in humans (see below). Other mycotoxins can also be found in milk, but generally their levels are much lower (Flores-Flores et al., 2015). Ochratoxin A can also accumulate in animal muscle and other organs, which can then cause adverse effects in humans when consumed as meat and meat products. Generally, most mycotoxins are heat tolerant within conventional food preparation and processing temperatures (80–121°C). Therefore, little or no reduction in feed or foodborne-mycotoxins occur as a result of cooking, boiling, and pasteurization (Scott, 1984; Smith et al., 1994; Kabak, 2009).

### *Aflatoxin $M_1$*

The International Agency for Research on Cancer (**IARC**) has classified $AFM_1$ as a group 2B carcinogen (IARC, 1993): a possible human carcinogen due to some evidence of carcinogenicity in laboratory animals though evidence in humans is insufficient. Some evidence exists that $AFM_1$, like $AFB_1$, can form DNA adducts in tree shrews and rats (Egner et al., 2003), but no such evidence has been found in humans. The Joint Expert Committee on Food Additives (**JECFA**) stated that the carcinogenic potency of $AFM_1$ in sensitive species is about one order of magnitude less than that of $AFB_1$ (JECFA, 2001).

For humans, the main source of exposure to $AFM_1$ in the diet is dairy products. In infancy, humans are also exposed through breastmilk (Khlangwiset et al., 2011). It is typically assumed that the concentration of $AFM_1$ in milk is one-fortieth (1/40) of the concentration of $AFB_1$ present in dairy animal feed. Therefore, in the United States, the action level for aflatoxin in dairy animal feed is 20 μg/kg to result in a maximum level of $AFM_1$ in dairy products of 0.5 μg/kg (FDA, 1994). Additionally, multiple other nations worldwide have set maximum tolerable levels of $AFM_1$ in dairy products (FAO, 2004) to minimize liver cancer risk from $AFM_1$.

### *Ochratoxin A*

Ochratoxin A is another mycotoxin that has been shown to occur in human foods through contamination of animal feed. However, it can be difficult to determine whether the OTA exposure in animal-source foods is due to the original contamination in animal feed, or due to contamination of the actual foodstuff during storage, transportation, or both. Regardless, it is known that OTA has a relatively long half-life in the body (Gilbert et al., 2001), and thereby has more potential for bioaccumulation.

Ochratoxin A is a renal toxin and carcinogen (JECFA, 2001). Although OTA has been implicated in multiple kidney-related diseases in experimental animals, there has been less human health evidence for adverse effects (Bui-Klimke and Wu, 2015). The IARC has classified OTA as a group 2B possible human carcinogen (IARC, 1993), based on evidence of renal carcinogenicity in experimental animals. Although, at the moment, no definitive study has linked human disease to dietary OTA exposure, it is important to consider that OTA could be a co-factor in increasing chronic kidney disease prevalence in human populations worldwide, with increasing rates of diabetes, overweight, and obesity (Chen and Wu, 2017).

For humans, the main source of dietary OTA exposure worldwide is cereal grains, followed by wine and grape juice, and then coffee and pork (JECFA, 2001). For comparative purposes, the average adult is exposed to 0.025 μg/kg of BW per wk of OTA through cereals, and to 0.0015 μg/kg of BW per wk of OTA through pork and pork products (JECFA, 2001). The JECFA set a provisional tolerable weekly intake of 0.1 μg/kg of BW per wk. It is possible that a significant portion of OTA contamination in pork is due to OTA contamination of silage in swine diets. In the United States, a recent survey of OTA in multiple foodstuffs gathered from grocery stores across the nation, OTA was detected in only 3 of 94 pork samples, at low levels (Mitchell et al., 2017). Compared with aflatoxin, few nations worldwide have set maximum tolerable levels for OTA in foodstuffs (Wu et al., 2014b). The maximum OTA limits proposed by Health Canada (Health Canada, 2009) and the European Union (European Commission, 2006, 2010) in raw cereal grains, derived cereal products, breakfast cereals, and dried vine fruits are 5, 3, 3, and 10 μg/kg, respectively.

## PREVENTING MYCOTOXIN CONTAMINATION OF SILAGES

### *Field or Preharvest Phase*

Preharvest control of mycotoxins is the first step to ensure that silages are safe. Mycotoxin contamination can be reduced by minimizing environmental stress on the plant through appropriate agronomic practices such as use of varieties or hybrids that are adapted to local conditions and are resistant to fungal infection, preventing insect and fungal infestation through application of fungicides and pesticides, proper management of crop residues and weeds, use of irrigation to prevent drought stress, timely crop rotation, and proper fertilization (Edwards, 2004; Whitlow and Hagler, 2005). Insect management is the most important practice for reducing the risk of mycotoxins, particularly fumonisins and aflatoxins, in temperate regions as insects act as vectors of fungal spores and insect damage predisposes crop plants, such as corn, to fungal infection (Munkvold, 2014).

The public health concern over the use of pesticides and fungicides as well as development of resistance to fungicides by fungal organisms has led to development of bio-agents to control mycotoxin contamination on the field (Tsitsigiannis et al., 2012). Application of atoxigenic strains of *A. flavus* or *Aspergillus parasiticus* to crops is a successful approach that has been used to reduce aflatoxin contamination on the field (Tsitsigiannis et al., 2010). Two atoxigenic strains of *A. flavus*, AF36 and NRRL 21882, are widely used in the United States to minimize aflatoxin contamination of crops (Tsitsigiannis et al., 2012). Recently, a bio-agent, known as aflasafe, that contains a mixture of 4 atoxigenic strains of *A. flavus* of Nigerian origin, was tested on corn plants in some African countries. Aflasafe treatment reduced aflatoxin concentrations of treated plants by over 80% compared with untreated plants (Bandyopadhyay et al., 2016).

### *Harvest Phase*

Careful harvest timing is important in controlling the level of mycotoxin contamination on forages. The risk of mycotoxin contamination is significantly reduced with earlier harvests (Jones, 1981). However, harvest should be timed to ensure optimum yield, DM concentration, and nutritive value.

The cutting height of the crop harvesters should be set to minimize soil contamination because of the widespread occurrence of *Fusarium* spores in soil (Jouany, 2007). Furthermore, immediate storage of harvested feeds is necessary to minimize the risk of mycotoxin contamination because delayed storage may expose the crop plants to temperature and humidity conditions that favor continued growth of toxigenic fungi and mycotoxin contamination (Munkvold, 2014).

### *Ensiling Phase*

Controlling mycotoxin production at the pre-harvest stage via physical, chemical, and biological methods is the best approach. However, the attendant problems with feed safety, potential losses in quality of commodities, limited efficacy, and cost implications limit success at this stage (Köhl et al., 2011). Therefore, complete control of mycotoxins at the pre-harvest stage cannot be guaranteed (Jard et al., 2011), which necessitates mycotoxin control during ensiling (Richard et al., 2009; Keller et al., 2013; Uegaki et al., 2013).

To minimize or prevent the growth of mycotoxins or undesirable microbes in silage, forages should be harvested at the recommended DM concentration or maturity stage and silos should be filled rapidly immediately after harvest with forage tightly packed to achieve recommended densities and completely sealed to preserve anaerobic conditions (Jouany, 2007).

Low silage pH and anaerobic conditions during ensiling inhibit the growth of spoilage and toxigenic fungi, and reduce production of mycotoxins in silage (Pahlow et al., 2003). Except for *Penicillium*, typical silage molds, such as *Fusarium*, *Aspergillus*, *Monascus*, *Rhizopus*, and *Geotrichum* species, are intolerant of low oxygen and low pH conditions (Pahlow et al., 2003). Therefore, during ensiling, molds are often found in poorly compacted areas such as the top layer or shoulders of silos, which are often more prone to oxygen infiltration than the rest of the silo. In addition, Gonzalez-Pereyra et al. (2008) noted that improperly managed silage due to poor compaction, air ingress, and low feed-out rate promotes aerobic conditions and less extensive fermentation that favors the growth of toxigenic molds that are normally less tolerant of acidic or anaerobic conditions. Once silos are opened, the aerobic conditions allow the growth of lactate-utilizing yeasts that metabolize lactate to $CO_2$ and, thereby, increase the pH (Muck, 2010). The increased pH and exposure to oxygen allow spoilage, pathogenic, and toxigenic fungi to grow aggressively during the feed-out phase (McDonald et al., 1991; Driehuis et al., 2018), particularly in poorly managed silages.

To reduce mold infestation or mycotoxin contamination (or both) during feed-out, aerobic stability-enhancing measures should be employed including maintaining a straight firm silo face, feeding out the silage at a minimum rate of 10 to 16 cm per day, and feeding the silage immediately after removal from the silo (Jouany, 2007).

Various studies indicate that sealing silos with oxygen-barrier plastic films can reduce oxygen penetration, mold infestation, and spoilage of silage (Borreani and Tabacco, 2008, 2012). Other studies have shown that such films can also limit aflatoxin production (Amaral et al., 2010; Cavallarin et al., 2011). These subjects are discussed in further detail by Borreani et al. (2018) in this issue.

In addition to the well-documented role of silage lactic acid bacteria in improving silage fermentation and aerobic stability and reducing fungal growth (Ranjit and Kung, 2000; Mari et al., 2009; Oliveira et al., 2017), some studies have examined their potential to mitigate mycotoxin contamination of silage in vitro (Gourama and Bullerman, 1995; Ahlberg et al., 2015). For instance, *Lactobacillus brevis* and *Lactobacillus plantarum* reduced ochratoxin content by 16.9 to 35% when grown in de Man, Rogosa, and Sharpe broth and by 14.8 to 26.4% in PBS after 24 h of incubation (Piotrowska, 2014). In a similar study, *L. plantarum* and *Lactobacillus paracasei* inhibited the growth of *F. graminearum* and reduced the concentration of DON by up to 67% after 24 h of incubation in de Man, Rogosa, and Sharpe broth (Franco et al., 2011). Recently, Martinez-Tuppia et al. (2017) isolated 2 lactic acid bacteria (*L. brevis* N195 and *L. brevis* N197) from ensiled high-moisture corn that degraded fumonisin $B_1$ into its hydrolyzed metabolite.

Only a few studies have used additives to reduce forage mycotoxin contamination. Inoculation of corn plants infested with southern rust with a mixture of *Pediococcus pentosaceus* and *Lactobacillus buchneri* at ensiling increased aerobic stability and prevented production of aflatoxins (5,200 vs. 0 μg/kg; Queiroz et al., 2012). In another study, *L. buchneri* inoculation reduced aflatoxin concentration but had no effects on concentrations of DON or ZEA in corn silage (Iglesias et al., 2005). Recently, Ma et al. (2017) assessed the capacity of lactic acid bacteria to bind $AFB_1$ in vitro and in corn silage that had been artificially contaminated with 30 μg/kg of $AFB_1$. The authors reported that certain silage bacteria can bind $AFB_1$ in vitro and the concentration of $AFB_1$ in the contaminated silage was reduced to a safe level within 72 h with or without lactic acid bacterial inoculation. Cavallarin et al. (2011) reported that limiting aerobic spoilage by inoculating corn silage with *L. buchneri* can reduce aflatoxin production. Teller et al. (2012) studied the effects of additive application and damaging ears of corn in the field before harvest on production of selected mycotoxins. Concentrations of DON and fumonisins were increased in damaged ears but not in undamaged ears and inoculation with *L. buchneri* 40788 (400,000 cfu/g of fresh forage) and *P. pentosaceus* (100,000 cfu/g), or treatment with potassium sorbate reduced mold and yeast counts and aerobic spoilage, but had no effect on the concentrations of DON, ZEA, and fumonisins in the corn silage. The concentration of fumonisins increased in corn forage treated with *L. buchneri* and ensiled for 60 d, though the concentration dropped to the initial level after 240 d of ensiling with or without additive treatment (Latorre et al., 2015). However, Boudra and Morgavi (2008) reported up to 50 to 100% reduction in the concentrations of fumonisin $B_1$ and DON in corn forage ensiled at low DM (24–28%) for 90 d, probably due to the high solubility of the toxins in the silage fluid, which might have been lost in the fermentation effluent (Lauren and Ringrose, 1997). However, in the latter study, both toxins, especially fumonisin $B_1$, were relatively stable (up to 70% stability) in corn forage ensiled at 36 to 42% DM for up to 180 d. In addition, the concentrations of other mycotoxins such as ZEA and fumonisin $B_2$ were relatively stable (up to 80% stability) in corn forage ensiled at 36% DM (Boudra and Morgavi, 2008), which is within the optimum DM range at harvest for whole-plant corn silage (Bal et al., 1997; Jouany, 2007).

The main deduction from these studies is that proper silage management and use of mold-inhibiting additives or microbial inoculants can decrease aerobic spoilage and reduce or prevent the growth of toxigenic fungi in silage (Kung et al., 2003). However, these measures cannot reduce concentrations of mycotoxins existing on plants before additive application, which occurs at harvest (Binder, 2007; Kristensen et al., 2010). In summary, good silage management principles and practices at all stages of silage making and feeding are essential to reduce mycotoxin contamination of silages.

## MYCOTOXIN DEACTIVATION BY RUMINAL MICROORGANISMS

Ruminants are more protected from toxic effects of mycotoxins than monogastrics because rumen microbiota can degrade or inactivate many of the toxic molecules (Pestka, 2007; Cheli et al., 2013; Gallo et al., 2015, 2016).

Kiessling et al. (1984) investigated the ruminal degradation of different mycotoxins and reported that whole rumen fluid degraded 90% of ZEA and 100% of T-2 toxin to their less toxic metabolites (β-zearalenol and HT-2 toxin, respectively), but had no effect on $AFB_1$. Furthermore, rumen microbes in cattle hydrolyzed 90% of dietary ZEA to α-ZEA (Kennedy et al., 1998). In another study, T-2 toxin, HT-2 toxin, and DON were completely degraded by rumen fluid in vitro when added at 10 μg/mL, but $AFB_1$ was not degraded (Westlake et al., 1989). The resistance of aflatoxin to ruminal degradation may due to the high inhibitory effect of aflatoxins on rumen microorganisms. For instance, 10 μg/mL of $AFB_1$ completely inhibited the growth of many ruminal bacteria (Jouany et al., 2009).

Most of the studies on the subject indicate that mycotoxin degradation was achieved primarily by ruminal protozoa (Jouany et al., 2009). Kiessling et al. (1984) reported that up to 100% of the degradation of ZEA and T-2 toxins were achieved by rumen protozoa. In addition, Hussein and Brasel (2001) reported that up to 90% of T-2 degradation was achieved by rumen protozoa. Therefore, protozoa are very important agents of mycotoxin biodegradation in the rumen (Upadhaya et al., 2010). However, the fact that protozoa cannot be cultured in vitro (Chaucheyras-Durand and Ossa, 2014; Newbold et al., 2015), limits further understanding of their ability to degrade mycotoxins. Studies involving culture-independent approaches based on analysis of particular genes and genomes potentially associated with mycotoxin degradation may help improve our understanding of the role of protozoa in the process.

Certain microorganisms in ruminal fluid notably *Eubacterium* strain BBSH 797 have been reported to degrade mycotoxins including mainly type A and B trichothecenes (T-2, HT-2, and DON), ZEA, and OTA (Westlake et al., 1987: Yang, 2010; Upadhaya et al., 2010, 2011). Fuchs et al. (2002) and Binder and Binder (2004) isolated and characterized *Eubacterium* strain BBSH 797 from rumen fluid due to its ability to detoxify and reduce the adverse effects of trichothecenes (DON and T2-toxin) in dairy cows. Upadhaya et al. (2011) speculated but did not confirm that *Bacillus licheniformis* is involved in degradation of ochratoxin in goat ruminal fluid can degrade ochratoxin.

The natural capacity of ruminant microbes to tolerate and degrade some mycotoxins can be saturated depending on the diet and or metabolic disease status (Fink-Gremmels, 2008) and feeding level or passage rate. For instance, starch-induced low ruminal pH (5.5 to 5.8) and high ruminal passage rates of high-producing cows may greatly reduce microbial detoxification of mycotoxins (Fink-Gremmels, 2008; Pantaya et al., 2016). This can allow a portion of some ingested mycotoxins, such as ochratoxins, to escape ruminal degradation and be transferred to animal products, particularly milk, posing a severe health hazard to humans (Shephard, 2008; Flores-Flores et al., 2015). Absorption of mycotoxins can take place throughout the gastrointestinal tract (Grenier and Applegate, 2013). However, most mycotoxins are absorbed in the proximal part of tract as evidenced by the prompt appearance of some ingested toxins in the blood (Cavret and Lecoeur, 2006; Grenier and Applegate, 2013). Aflatoxins, particularly $B_1$, are absorbed at a high rate in all livestock species (Grenier and Applegate, 2013) and some of the absorption may occur in the mouth or esophageal membrane of dairy cows (Gallo et al., 2008). Absorption of other ingested mycotoxins, such as trichothecenes, ochratoxins, and fumonisins can vary from 1 to 60% (Cavret and Lecoeur, 2006; Bouhet and Oswald, 2007; Grenier and Applegate, 2013). However, effects of mycotoxins on conditions in the abomasum are unknown.

## MYCOTOXIN DETOXIFICATION STRATEGIES

Strategies for detoxification of mycotoxins are aimed at destroying or reducing the concentration of a mycotoxin, or making it nontoxic and or unavailable for absorption in the gut of ruminants (CAST, 2003). Despite several efforts to prevent or minimize the occurrence of mycotoxins at pre- and postharvest stages, the current control strategies are not effective enough to guarantee complete detoxification or elimination of mycotoxins or reduction of their concentrations to safe threshold levels. Consequently, frequent instances of feed contamination with mycotoxins occur.

Though effective for other feeds, physical methods such as manual removal, cleaning, or milling of grains contaminated with fungi (Bata and Lásztity, 1999; Siwela et al., 2005), physical methods such as thermal irradiation (Raters and Matissek, 2008), and chemical methods such as ammoniation (Hoogenboom et al., 2001) and ozonation (McKenzie, 1997) are too costly and ineffective for ensiled feeds because of their high bulk or volume.

### *Dilution*

Dilution of contaminated silage with other ingredients in rations is an effective strategy for reducing the ingestion of mycotoxins, though it does not detoxify the toxin. However, it is not an ideal strategy for contaminated silage because it can reduce the feed-out rate, which may promote further mold growth and mycotoxin production (Whitlow and Hagler, 2005). Consequently, dilution is not a recommended strategy for mycotoxin mitigation in Europe (Siegel and Babuscio, 2011).

### *Adsorption*

A feasible, safe, and relatively cost-effective alternative strategy to reduce problems associated with ingestion of mycotoxins is to decrease their bioavailability in the gut. This involves adsorption of the toxin by sequestering agents in the diets (CAST, 2003).

Adsorption mechanisms comprise binding by minerals and or organic compounds or biological adsorption. The capacity of several of these adsorbents to decrease the bioavailability of certain mycotoxins has been reviewed (Avantaggiato et al., 2005; Jard et al., 2011). These adsorbents can be used in specific matrixes such as silages, to reduce mycotoxin uptake from the gastrointestinal tract into the blood and target organs (Jard et al., 2011). However, the efficacy of these adsorbents is dependent on the mycotoxin and environmental factors (Jard et al., 2011; Faucet-Marquis et al., 2014) as well as the diet, animal performance level, and management. Several adsorbents can bind aflatoxin, but few have been effective at binding other mycotoxins (Boudergue et al., 2009), particularly DON.

***Clay Adsorbents.*** The use of clay minerals as mycotoxin-sequestering agents in livestock feeds has been well documented (Stroud, 2006). Clay minerals are fine-grained components of geological materials, occurring as particles with a phyllosilicate structure formed usually as products of chemical weathering (Velde and Menier, 2008). Clay minerals can hold water and dissolved nutrients due to their unbalanced electrical charges (Velde and Menier, 2008); therefore, they can adsorb organic substances on their external surfaces or within their inter-layer spaces by the interaction or substitution of the exchanged cations within these spaces (Velde and Menier, 2008). Aflatoxins can be adsorbed into this porous structure and get trapped by electric charges (Jouany, 2007); consequently, adsorbent clay minerals are effective at binding $AFB_1$ in liquid media (Moschini et al., 2008) and in cow diets. Kutz et al. (2009) examined the efficacy of 2 calcium montmorillonite clay-based sequestering agents in mid-lactation cows fed 100 μg/kg of $AFB_1$ and reported they reduced the milk $AFM_1$ concentration by 45 and 48%, respectively. Queiroz et al. (2012) evaluated the effects of dietary addition of 0.5 and 1% of diet DM of a montmorillonite clay-based mycotoxin sequestering agent and reported that the high dose reduced the concentration of $AFM_1$ in the milk. Furthermore, Maki et al. (2016) demonstrated that montmorillonite clay (1% of diet DM) reduced the milk $AFM_1$ concentration and had no negative influence on feed intake, mineral, vitamins, and milk production of dairy cows. Although, clay-based products have been effective at adsorbing aflatoxin in diets, their composition varies significantly by lot and type; therefore, the efficacy and safety of each product should be evaluated before inclusion in animal diets (CAST, 2003). The most extensively studied clay minerals are a range of products known as hydrated sodium calcium aluminosilicate (**HSCAS**), and montmorillonite is one of those frequently used (Jouany, 2007). This clay mineral binds with aflatoxin by chelating the β-dicarbonyl moiety in aflatoxin with uncoordinated metal ions in the clay materials (Phillips et al., 2002; Whitlow and Hagler, 2005). Hydrated sodium calcium aluminosilicate has high affinity for $AFB_1$ (Phillips et al., 2002). In fact, the aflatoxin-HSCAS binding complex formed is stable over a wide pH (2–10) and temperature (25–37°C) range (Phillips et al., 2002). Other clay products that have been studied include bentonites, zeolites, clinoptilolites, and others that are not characterized (Whitlow and Hagler, 2005). Clay minerals differ in terms of ion exchange capacity, surface area, and particle size, shape, and distribution (Grim, 1968). Montmorillonite clay is believed to have the greatest cation exchange capacity (Grim, 1968). Due to such compositional differences, it is important for research studies to report the characteristics of clay products being tested.

Some of the limitations of clay-based products as mycotoxin sequestering agents are that they accumulate in manure, may be contaminated with toxic metals and dioxins, and are effective at binding aflatoxins but not other mycotoxins (Bursian et al., 1992; Phillips et al., 2002). For instance, the inclusion of HSCAS in the DON-contaminated diet of pigs did not mitigate the effects of DON (Patterson and Young, 1993). Also, HSCAS reduced the toxicity of aflatoxin, but did not prevent that of OTA alone or combined with aflatoxin in poultry (Huff et al., 1992). In addition, clay-based sequestering agents can interfere with mineral absorption. Chestnut et al. (1992) reported impaired absorption of zinc, magnesium, and manganese in sheep fed HSCAS. Similar findings were observed in poultry (Chung et al., 1990) and swine (Schell et al., 1993). No published reports indicating that clay-based sequestering agent ingestion impaired mineral absorption in dairy and beef cattle were found. However, a recent study reported an increased loss in milk yield as concentration of clay product increased from 0.5 to 2% in dairy cows fed 100 μg/kg of dietary aflatoxins (Sulzberger et al., 2017). The clay product used in this study contained vermiculite, nontronite, and montmorillonite.

***Activated Charcoal.*** Activated charcoal is a mycotoxin sequestering agent with a large surface area and excellent adsorptive capacity (Whitlow and Hagler, 2005). However, the effects of activated charcoal on dietary mycotoxins have been variable. Galvano et al. (1996) reported that 2 activated charcoals differing in surface area and iodine number were fed at 2% of the diet and reduced the transfer of $AFB_1$ into the milk of cows fed 11 μg/kg by 22 to 45%, but these responses were not better than that with HSCAS. However, when cows were fed 55 μg/kg of $AFB_1$, feeding 45 g per cow daily of activated charcoal did not reduce milk $AFM_1$ residues, whereas bentonite clay (225 g per cow daily) or an esterified glucan (10 g per cow daily) did (Diaz et al., 2004). This suggests that the efficacy of activated charcoal is dependent on the level of contamination by the toxin. Studies with broilers (Edrington et al., 1997) and turkey poults (Edrington et al., 1996) also suggest that charcoal is not as effective as clay-based sequestering agents.

***Polyvinyl Pyrrolidone.*** Polyvinyl pyrrolidone (**PVP**), a synthetic water-soluble polymer, has been effected as a ZEA-sequestering agent. Alegakis et al.

(1999) reported binding of ZEA by PVP ranging from 33.5 to 66.2% per 25 mg of PVP in an in vitro medium. However, no studies on the binding efficacy of PVP in animals were found.

***Yeast-Based Adsorbents.*** Microorganisms and or their products have been evaluated as mycotoxin sequestering alternatives to nonnutritional adsorbents in the diet. Yeast and yeast cell wall products have been evaluated for their ability to bind aflatoxins and other mycotoxins in several studies (Devegowda et al., 1998). Sequestration of mycotoxins in vitro and in vivo by yeast is caused by adhesion of the toxin to the major cell wall components (mannan and β-glucan; Pfliegler et al., 2015), which are also nutrient sources and growth promoters (Fruhauf et al., 2012). Beta-glucans (glucomannan) fed at 0.05 or 0.1% of diet to dairy cows or poultry, respectively, bound aflatoxins, ochratoxin, and T-2 toxin (Raju and Devegowda, 2000; Diaz et al., 2004). Furthermore, Freimund et al. (2003) noted that a glucan polymer bound both T-2 toxin and ZEA in vitro. An additional benefit of adding yeast or yeast products to diets is that β-glucan can improve the performance of animals by stimulating both nonspecific and specific immunological responses (Keller et al., 2015). A yeast cell wall product tested on dairy cows reduced the $AFM_1$ concentration of milk by 59% when fed at 0.05% of a diet contaminated with 55 μg/kg of $AFB_1$ (Diaz et al., 2004). However, no effects of yeast products were found in diets contaminated with 122 (Kutz et al., 2009) or 80 μg/kg (Kissell et al., 2013) of $AFB_1$, indicating that the efficacy of yeast products is probably dependent on the extent of mycotoxin contamination.

***Chlorophyll-Based Adsorbents.*** Chemoprotection against aflatoxins has been demonstrated with the use of chemical compounds such as chlorophyllin, a chlorophyll-based product. The mechanism of action of chlorophyll-based product involves trapping $AFB_1$ and other carcinogens via binding of their planar ring structures to that of chlorophyllin (Arimoto et al., 1993), thereby preventing the toxin from forming adducts with DNA, and hence facilitating the clearance of the toxin from the body (Hsu et al., 2008). The binding effects of chlorophyllin on $AFB_1$ also have been studied in vitro (Hsu et al., 2008). Hsu et al. (2008) reported that treatment of murine hepatoma cells with 2 chlorophyll derivatives, chlorophyllide and pheophorbide, significantly reduced the formation of $AFB_1$-DNA adducts via direct trapping of $AFB_1$. In humans challenged with 30 ng of $AFB_1$, 2 chlorophyll products fed at 150 mg daily reduced excretion of urinary aflatoxin equivalents by 40 to 60% (Jubert et al., 2009). No published studies were found on the efficacy of using chlorophyll derivatives alone to sequester mycotoxins in dairy cow diets.

***Combination Adsorbents.*** Different adsorbent products have been combined to improve the mycotoxin-binding efficacy of the component products. Unfortunately, studies comparing the efficacy of the combined product to that of its components in dairy cows are rare. A few studies have attempted to validate such combination products in dairy cows. Xiong et al. (2015) reported that adding a blend of sodium montmorillonite with live yeast, yeast culture, and mannan oligosaccharide fed at 0.25% of diet DM reduced transfer of $AFB_1$ from diets contaminated with 20 μg/kg of $AFB_1$ into milk but had no effect when diets were contaminated with 40 μg/kg of $AFB_1$. In a recent study, Ogunade et al., (2016) examined the effects of a sequestering agent product containing a mixture of *Saccharomyces cerevisiae*, clay and chlorophyll-based products in dairy cows challenged with 1,725 μg of $AFB_1$. The sequestering agent had no effect on the transfer of the toxin into milk, probably because the low dose (20 g/d) supplied insufficient clay (0.05% DM equivalent), but it prevented the inflammatory stress response and anemic conditions observed in cows fed the toxin alone. Feeding 200 g of a clay product together with a 35 g of yeast product reduced the transfer of the $AFB_1$ into milk, improved the performance of the cows, and prevented potential liver damage in cows fed 1,725 μg of the toxin daily for 5 d (Jiang et al., 2018). A combination product containing mycotoxin-degrading enzymes and mineral adsorbents improved milk production and prevented mild hepatocellular injury in dairy cows fed diets naturally contaminated with up to 10 μg/kg of $AFB_1$, 1,000 μg/kg of ZEA, and 600 μg/kg of DON (Jovaisiene et al., 2016). A similar result was reported by Kiyothong et al. (2012).

### *Mycotoxin Detoxification by Exogenous Microorganisms*

Microbial degradation is a potentially promising, environmentally friendly strategy of mycotoxins in feed and foods due to irreversibility of the catalyzed reactions (Kolosova and Stroka, 2011). The degradation of mycotoxins into nontoxic or less toxic metabolites compared with the parent toxin can be achieved directly by enzymes or microorganisms that produce such enzymes (Loi et al., 2017). Bacteria, yeast, fungi, and enzymes that degrade mycotoxins have been isolated from different sources (Guan et al., 2009, Upadhaya et al., 2012). For instance, Guan et al. (2009) reported that a microbial culture from fish digesta degraded DON over a broad range of temperatures (4 to 25°C) and pH values (4.5 to 10.4) under aerobic conditions. Motomura el al. (2003) reported that *Pleurotus* sp.

degraded $AFB_1$ in cultures at 25°C and pH 4.0 to 5.0. Similarly, Teniola et al. (2005) showed that cell-free extracts from *Rhodococcus erythropolis* and *Mycobacterium fluoranthenivorans* sp. nov. DSM44556 degraded $AFB_1$ at temperatures ranging from 10 to 40°C. Their efficacy at mycotoxin degradation by such organisms and the associated mechanisms have been comprehensively reviewed by several authors (Kolosova and Stroka, 2011; McCormick, 2013; Vanhoutte et al., 2016; Loi et al., 2017). Recently, Martinez-Tuppia et al. (2017) isolated *Lactobacillus* strains from high-moisture corn grain silages that could convert fumonisin $B_1$ to the less toxic hydrolyzed fumonisin $B_1$ metabolite. Such strains may also be effective at degrading fumonisin $B_1$ in grass, legume, or whole crop-cereal silages. More research is needed to identify other silage microbes that can degrade or detoxify mycotoxins.

The efficacy of microbial or enzymatic mycotoxin degradation depends on the enzyme or microorganism and environmental parameters including pH, temperature, availability of nutritive components, microorganism concentration, and availability of oxygen and other substrates (He and Zhou, 2010). He et al. (1992) showed that degradation of DON to its deepoxy form by both rumen and microbial inoculum from intestines of broilers was inhibited when the medium pH dropped to 5.2 but was unaffected by the initial concentrations of DON (14 to 1,400 μg/mL) and the microbial inoculum (0.2 to 0.8 g/mL).

Guan et al. (2009) reported that *Stenotrophomonas* sp. degraded $AFB_1$ to a greater extent at pH 8 (85%) than at pH 4 (14%). Thus, the reduction in pH produced by accumulation of silage fermentation products may inhibit complete degradation of $AFB_1$.

Other environmental factors, such as ion concentration, may influence mycotoxin degradation. Sangare et al. (2014) reported that $AFB_1$, $AFB_2$, and $AFM_1$ degradation was strongly affected by metal ions, with $Mg^{2+}$ and $Cu^{2+}$ behaving as activators and $Li^{+}$, $Zn^{2+}$, $Se^{2+}$, $Fe^{3+}$ 450 being strong inhibitors. Hartinger and Moll (2011) reported that during fumonisin $B_1$ degradation by *Sphingopyxis* sp. MTA144, the bacterial aminotransferase encoded by the gene Fum I worked best at low salt concentrations under a wide range of pH (6 to 10) and temperature (6 to 50°C). The aforementioned studies underscore the important influence of environmental factors in modulating mycotoxin degradation by exogenous microbes. Although some of the microbial agents may be effective at silage and ruminal pH values, others will not. Isolation of candidate mycotoxin-degrading microorganisms from a matrix is more likely to ensure that they will be effective under the typical conditions to which the matrix is exposed (Zhu et al., 2017). Hence, more attention should be paid to isolating mycotoxin-degrading microbes and enzymes from silage and the rumen.

### *Mycotoxin Detoxification by Exogenous Natural or Recombinant Enzymes*

Loi et al. (2017) emphasized that using whole-cell living microorganisms to deactivate mycotoxins could be costly due to the multi-step reactions required for feed additive or industrial applications. Microbes may also have limited efficacy in environments with high mycotoxin concentrations. Detoxification of mycotoxins by specific microbial enzymes may represent a suitable alternative strategy to increase feed and food safety (Wu et al., 2009; Hartinger and Moll, 2011; Loi et al., 2017). For instance, Liu et al. (1998) reported the extraction and characterization of an enzyme from *Armillariella tabascens* that completely detoxified 16 μ*M* of $AFB_1$. In another study, extracellular extracts from *Rhodococcus erythropolis* liquid cultures enzymatically degraded 66.8% of $AFB_1$ and prevented mutagenicity of the toxin (Alberts et al., 2006). Abrunhosa and Armando (2007) isolated an enzyme that hydrolyzed OTA from *A. niger*. Similarly, carboxypeptidase A in *Phaffia rhodozyma* degraded 90% of OTA (Peteri et al., 2007). However, one major challenging aspect of this strategy is the difficulty of achieving uniform enzyme delivery throughout the entire material (Loi et al., 2017), which is a particular problem for silages.

Heinl et al. (2010) provided a basis for the development of an enzymatic detoxification process for fumonisin $B_1$ in food and animal feeds, especially under oxygen-limited conditions, as in silages. However, part of this degradation process by microbial enzymes requires co-substrates and specific conditions that are not naturally present. Genetic engineering may allow tuning of detoxification enzymes to ensure their activities are adapted to the target environment (Hartinger and Moll, 2011). Takahashi-Ando et al. (2002) reported a lactonohydrolase encoded by gene zhd101 from the fungus *Clonostachys* spp., responsible for ZEA degradation. This enzyme was purified and the entire coding region of zhd101 was cloned into the heterologous hosts, *Schizosaccharomyces pombe* and *Escherichia coli* and ZEA degradation activity by these host yeasts was confirmed. This was a promising approach to degrading ZEA using microbes adapted to the target environment. In a later study, Takahashi-Ando et al. (2004) expressed an enhanced green fluorescent protein (*EGFP*) gene fused to zhd101 (egfp::zhd101) to visually monitor lactonohydrolase protein. Whereas recombinant *E. coli* and transgenic rice calluses exhibited strong *EGFP* fluorescence and complete ZEA degradation, recombinant *S. cerevisiae* exhibited poor fluorescence and removed only 75% of ZEA. Based on these results, Igawa et al. (2007) expressed the egfp::zhd101 gene in corn kernels and resulted in ZEA degradation. Popiel et al. (2014) reported the first finding of functional ZEA lactonohydrolase in a recombinant mycoparasite, *Trichoderma aggressivum*. Therefore, an appropriate combination of a candidate host organism and a codon-optimized synthetic gene strategy may contribute significantly to establish a mycotoxin detoxification system for food and feed (Takahashi-Ando et al., 2004). In addition, the expression of suitable heterologous genes offers a practical approach for developing microorganisms with endogenous transgenic detoxifying activities (Karlovsky, 1999). Therefore, the expression of some adapted microbial detoxification genes in plants may limit pre-harvest mycotoxin accumulation and protect crop plants from the phytotoxic effects of mycotoxins (McCormick, 2013). The advantages of this strategy are obvious from both economic and ecological points of view, particularly as no chemicals or inocula are required (Karlovsky, 1999). Nonetheless, the main problem with enzymatic inactivation of toxins in engineered crops may be the inaccessibility of the toxins to the degrading enzymes (Karlovsky, 1999).

Various mycotoxin-detoxifying genes have been expressed in different plants with variable successes as reviewed by Karlovsky (2011) and McCormick (2013). However, public and scientific concerns related to the use of genetically modified plants as food or feed ingredients make this approach less suitable than others for mycotoxin control (Varga and Tóth, 2005). Therefore, naturally produced enzymes are still the best strategy to deactivate mycotoxins in food and feed industries, particularly when they are optimized and produced with high yield in microbial cell factories (Hartinger et al., 2010). Industrial production of detoxifying microorganisms involves their growth in large batches with optimal conditions leading to specific enzymatic production, followed by extraction and purification (Upadhaya et al., 2010). Nevertheless, to date, only a few enzymes have been purified, characterized and validated for their ability to degrade mycotoxins in in vitro and in vivo assays (Loi et al., 2017). Another challenge related to using mycotoxin-degrading enzymes is mycotoxin co-occurrence. Therefore, combining different degradation strategies, such as adsorbent mixtures, binding microorganisms and enzymes, may be more effective (Loi et al., 2017).

## FUTURE RESEARCH PRIORITIES

Due to the ubiquitous occurrence of mycotoxins in grains and forages, frequent contamination of livestock feeds with multiple mycotoxins, inconsistent effects of available sequestering agents and the resulting global threat to food and feed safety and security, future research should aim to develop more effective mycotoxin-sequestering agents that can counteract the additive or synergistic effects (or both) of multiple mycotoxins. Because silages contribute substantially to mycotoxin contamination of livestock feeds, concerted research efforts should aim to identify or develop silage additives that are effective at increasing the fermentation, nutritional value, and aerobic stability of silage but that also sequester or degrade silage mycotoxins. These microbes should have the potential to survive the ensiling process and rumen conditions, so they can contribute to ruminal degradation of mycotoxins from other feed ingredients. Furthermore, studies on the ecology of specific rumen microbes that degrade mycotoxins, particularly aflatoxins, are also needed to reduce their toxic effects and transfer to human foods. Jouany (2007) suggested that identifying and studying the ecology of specific gut microbes that can degrade mycotoxins in the rumen and creating enabling growth conditions for these microbes in the gut is an important strategy to overcome the problem of some mycotoxins. The inability to quickly and accurately diagnose mycotoxicoses is an important problem. Future studies should aim to develop tools for accurate diagnosis of mycotoxicoses in ruminants. Lastly, to replace expensive chromatography methods, hazardous RIA methods, and laborious ELISA methods, future research also should develop simple, rapid, and accurate mycotoxin detection techniques to foster on-site or on-farm detection of mycotoxins in feeds.

## CONCLUSIONS

Problems associated with mycotoxins in silage can be minimized by preventing fungal growth before and after ensiling. Therefore, proper silage management and use of mold-inhibiting chemical additives or microbial inoculants are essential to reduce mycotoxin contamination of livestock feeds. This review has highlighted the need for new studies that develop silage epiphytic microbes and potent but safe chemicals into novel silage additives that improve silage fermentation and storage but also effectively degrade silage mycotoxins.

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