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# Survey of Mycotoxins Present in Florida Pastures Across Time, Locations, and Grass Species[1]

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## Purpose and Audience

Warm-season pasture grasses are hosts to fungal endophytes that can produce various secondary metabolites. These grass fungal endophytes live in the grass tissues, and most grasses typically do not exhibit any visible symptoms. The secondary metabolites produced by these fungi are organic compounds that are not directly involved in fungal growth, development, or reproduction. Instead, these compounds are often produced in response to environmental stressors or as part of the fungal interaction with the grass hosts. Some of these compounds may adversely affect the livestock that consume them. Recently, local ranchers raised concerns about the consumption of mycotoxins (a type of fungal-produced secondary metabolites) as a potential detriment to livestock health. Since 2017, our team has been investigating the distribution of mycotoxins across these pastures in Florida. This publication presents findings on mycotoxins detected in the state's predominant pasture grass species, including bahiagrass, limpograss, and bermudagrass. Our goal is to provide information about the types and concentrations of mycotoxins to livestock producers, Extension professionals, and others interested in the impact of these substances. The insights provided will enhance the understanding of mycotoxin presence in Florida's warm-season pastures, supporting producers and their veterinarians in effectively addressing livestock health concerns.

## Introduction

Mycotoxins are toxic compounds produced by certain fungal groups that can cause disease and death in humans and animals. The toxicity of grasses caused by mycotoxins has led to economic losses in the United States due to the health impact on the livestock feeding on it. As witnessed with tall fescue toxicosis, the annual economic loss has increased largely from $1.5 billion to $3 billion. In addition, the changing climate with more extreme stress events (e.g., global warming) also favors growth of the causal

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agents of mycotoxins—the grass fungi, including both the endophytes that live in plant tissues, such as *Balansia*, and epiphytes that reside on the surface plant tissues, such as *Myriogenospora* (Chen et al. 2022). Florida has a warm and humid climate, and such conditions are very favorable for the growth of these grass fungi. In recent years, Florida cattle ranchers reported some health issues with their herds related to grazing on the grass pastures, such as bahiagrass, limpograss, and bermudagrass. Generally, the forage in Florida is largely palatable. However, certain weather conditions can promote the growth of some fungi in these grasses, often inducing the production of mycotoxins. Some of these "mycotoxins," when present at certain concentrations, can lead to the sickness of the animals that graze on them. The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) has provided an evaluation for some high-risk mycotoxins in food and feed worldwide, which are also present in Florida pastures, such as ochratoxin, zearalenone, and fumonisins. The JECFA has also established risk management advice to prevent contamination by some of these mycotoxins in food. However, more research is needed to determine the optimal thresholds (maximum levels) of these mycotoxins that may lead to impacts on the overall health and well-being of our livestock.

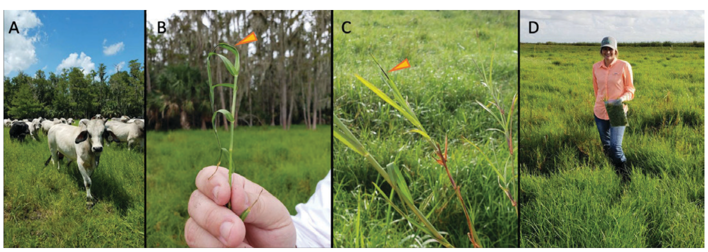


Figure 1. The representative field sites for the samples of this report. (A) The bulls in the ranch of Brevard County, Florida. (B and C) Myriogenospora atramentosa formed black stroma (arrows) on these limpograss leaves, which were found at the collection sites (Brevard County [B] and Osceola County [C]) (Chen et al. 2019). (D) Brittany Justesen, county Extension agent, collected the forage samples for mycotoxin analysis.
Credits: Brittany Justesen, UF/IFAS

Except in a few cases (e.g., *Myriogenospora atramentosa* forming black stroma on leaves: Figure 1), fungi living in grass leaves ("endophytes") or living on grass leaves ("epiphytes") typically do not cause any visible symptoms in the grasses. Thus, we largely rely on lab work to test the abundance of these fungi and the level of mycotoxins they produce. Generally, we lack information about the nature of mycotoxins that are present in Florida pastures across different environmental conditions. This information is important as it can allow ranchers to fine-tune their management strategies to prevent animals from consuming grasses with relatively higher concentrations of mycotoxins. Through the efforts of UF/IFAS Extension agents who collected grass samples starting in 2017 over a few years (Figure 1), we were able to successfully collect and examine 195 grass samples (four grass species) from 13 ranchers across dry and wet seasons in Florida (Figure 2). In this publication, we summarize the results and provide our ranchers with detailed information on (1) what (dominant) mycotoxins are generally present in Florida's pastures, (2) what grass species could harbor more of these mycotoxins, and (3) what seasons or locations may favor these dominant mycotoxins.

## The Dominant Mycotoxins Identified from 13 Ranches Across Florida

In this report, we highlight the top mycotoxins identified in the 195 grass samples collected from north (NR), central (CR), and south (SR) Florida ranches (Table 1). It seems that geographic location can be associated with the accumulation of dominant mycotoxins (Figure 2). The most dominant mycotoxins found across ranches in central and south Florida were zearalenone-4-sulfate (produced by *Fusarium* spp.). Higher concentrations of zearalenone-4-sulfate were mostly found in central Florida. Dihydrolysergol (produced by *Aspergillus*, *Penicillium*) was found in north Florida ranches (NR2 and NR4). Alternariol methyl ether (produced by *Alternaria* spp.) was identified as the top mycotoxin in NR3. Emodin (produced by grasses themselves or fungi) was the top metabolite (i.e., the small molecule produced by metabolisms) in terms of prevalence and concentration in some ranches across Florida.

## Mycotoxins and Their Causal Agents (Fungi)

*Fusarium* spp. that produce zearalenone and its derivatives, such as zearalenone-4-sulfate, are found in a wide range of environments and are known to infect a variety of crops, including cereals (e.g., corn, wheat, barley) and other plants (e.g., sugarcane, rice, sorghum) (Mousavi Khaneghah et al. 2019; Weidenbörner 2017). Based on the maximum tolerable intake levels established by the JECFA (0.5 ppb) and Dairy One (10 ppb), which are considered harmful to cattle, our analysis revealed that zearalenone concentrations in 33.3% and 26.7% of the collected grass samples exceeded these thresholds, respectively (Table 2). This issue is particularly notable in most ranches across central and south regions, including SR2 (65%), CR1 (50%), and CR6 (75%), based on the JECFA standard (Figure 3). In response, we shared the reports with ranchers from CR and SR ranches this year to raise awareness. Cattle may graze randomly on these grasses and this mycotoxin may degrade in their rumen, potentially keeping their overall zearalenone intake below harmful levels; however, even moderate levels that do not cause clinical symptoms can still impair cattle performance, resulting in economic losses. Therefore, further investigation of zearalenone accumulation in cattle beef by veterinarians is essential to assess its direct impact on cattle health. Zearalenone toxicity leads to specific symptoms in animals (Gott et al. 2018; Zinedine et al. 2007; Patriarca and Pinto 2017). For example, in ruminants, zearalenone can cause reproductive disorders, including infertility and reduced milk production, and alter levels of estrogen and other hormones that lead to changes in the development and growth of animals (Gott et al. 2018; Zinedine et al. 2007). This data report could provide valuable information for veterinarians, enabling them to correlate symptoms observed in cattle from these or nearby ranches with zearalenone exposure.

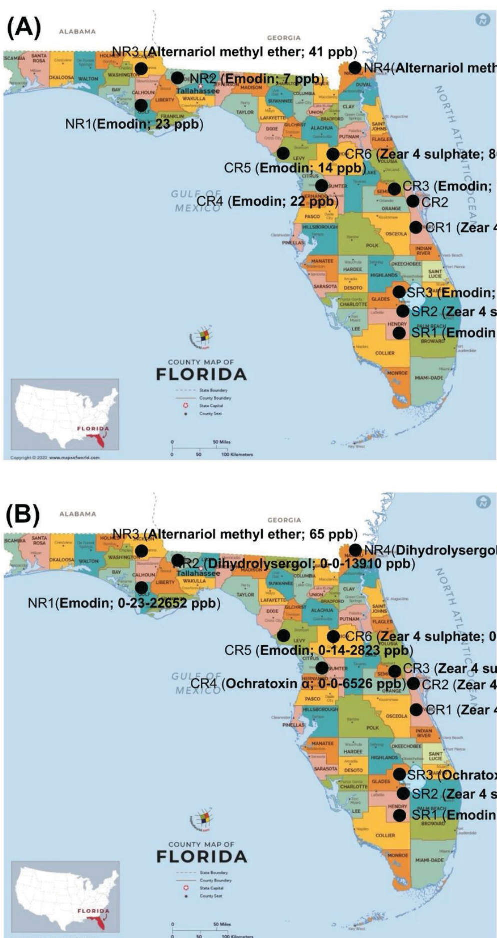


Figure 2. The locations of ranches across Florida and the most dominant mycotoxin (shown in bold) of grass leaves in each ranch. (A) The highest median concentration of toxins. (B) The prevalence (minimum-median-maximum) of the highest mean concentration of toxins (ppb).
Credits: Kaile Zhang, UF/IFAS

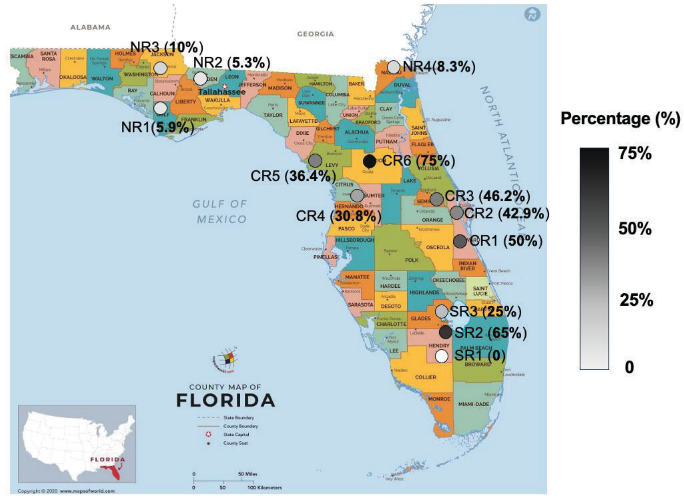


Figure 3. The percentage of grass samples containing zearalenone that are higher than the maximum tolerable intake level harmful to cattle in each ranch as suggested by JECFA.
Credits: Kaile Zhang, UF/IFAS

Alternariol is a mycotoxin produced by certain *Alternaria* spp., which is commonly found in a wide range of agricultural products, such as vegetables, cereals, and nuts (Solhaug et al. 2016). Alternariol has been found to have toxic effects on animals and humans, as well as genotoxic and cytotoxic properties (den Hollander et al. 2022; Terminiello et al. 2006). For example, alternariol can suppress the immune system, making animals more susceptible to infections, and can cause liver and kidney damage in animals (den Hollander et al. 2022).

Ochratoxin alpha is primarily produced by *Aspergillus* spp. and *Penicillium* spp. (Welke 2019). It is commonly found in food and feed products stored under humid and warm conditions, such as cereal-based products, coffee, grapes, and dried fruits (Sorrenti et al. 2013). Some studies showed that cattle can degrade ochratoxin alpha in feeds contaminated with up to 12 ppm, but this ability depends on the functionality of the rumen (Battacone et al. 2010). However, ochratoxin alpha can still be found in the meat and milk of cows in some cases as a result of consuming contaminated feed (Marquardt and Frohlich 1992). In monogastric animals such as pigs, ochratoxin alpha can cause kidney damage and cancer, as well as suppress the immune system, making them more susceptible to infections (Pfohl-Leszkowicz and Manderville 2007).

Dihydrolysergol is a derivative of the mycotoxin lysergic acid, which is produced by the *Claviceps* spp. and is known to have psychoactive effects that refer to changes in perception, mood, consciousness, behavior, or cognition (Bragg 2017). Dihydrolysergol is typically found in cereal-based products, especially in rye and rye-based products, and can infect other grasses as well (Iqbal et al. 2016).

The causal agents (fungi) (*Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., and *Claviceps*) of these toxins thrive in warm and humid conditions. For example, the optimal growth for *Fusarium* spp. is at temperatures of 77°F–86°F and relative humidity of 85%–90% (Di Menna et al. 1991; Jimenez-Garcia et al. 2018). Accordingly, high humidity, prolonged leaf wetness, and moderate temperatures are ideal growth conditions for *Fusarium* spp. that produce zearalenone. It is possible that the samples collected from central Florida ranches (i.e., CR1, CR2, CR3, and CR6) were taken during conditions that favored the production of zearalenone by *Fusarium* spp. Similarly, alternariol production by *Alternaria* spp. is favored by warm and humid climates (i.e., 68°F–86°F and 85%–90% relative humidity) (Solhaug et al. 2016). Moreover, high temperatures (68°F–95°F) and relative humidity (80%–100%) can result in a high concentration of ochratoxin alpha produced by *Aspergillus* spp. and *Penicillium* spp. (Paterson et al. 2018; Abarca et al. 2019).

Emodin is a naturally occurring compound that can be found in various plants, including rhubarb, buckthorn, and senna. Emodin can also be produced by fungi (e.g., *Aspergillus* spp., *Penicillium* spp., or in its sexual state, *Taraomyces* and *Fusarium* spp.) (Izhaki 2002; Fukaya et al. 2022). Emodin can have some beneficial properties for plants, such as helping protect plants against pathogenic microbes and herbivores (Dong et al. 2016; Semwal et al. 2021; Sevilla et al. 2007). However, emodin may have toxic effects on the liver and kidneys, particularly when consumed in high doses or over a long period of time (Dong et al. 2016). Emodin was found to be the dominant metabolite present in Florida's bahiagrass when the grass was affected by *Myriogenospora atramentosa* (Chen et al. 2022).

## Common Mycotoxins Present in Pasture Grass Species of Florida

Our previous study showed that the aforementioned mycotoxins (beta-zearalenone, alternariol methyl ether, dihydrolysergol) were the dominant mycotoxins present in bahiagrass leaves across Florida pastures (Chen et al. 2022), but their concentrations were relatively low in bahiagrass, with the level of 0.01 ppb for beta-zearalenone, 14 ppb for alternariol methyl ether, and 15 ppb for dihydrolysergol. Similar to our previous study, we found that the concentrations of the top toxins in bahiagrass were relatively lower compared to other examined grass species (Figure 4).

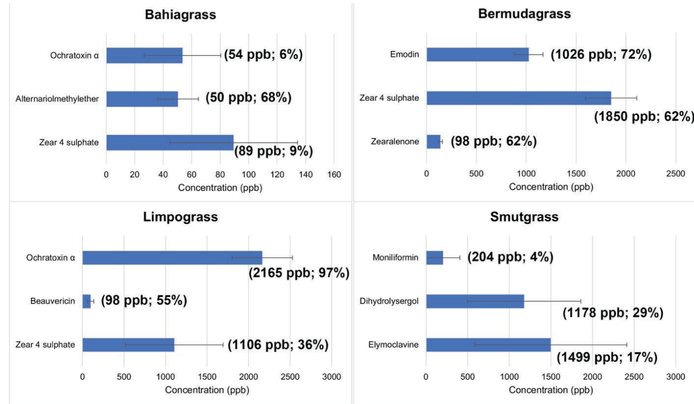

Figure 4. The most dominant mycotoxins (top 3) in different grass species across Florida. The values and percentages in the parentheses of each graph represent the average concentration of each dominant mycotoxin and its prevalence, respectively. Error bars indicate standard errors of each variable (Mendez 2022).
Credits: Kaile Zhang, UF/IFAS

Among 195 grass samples collected in this report, 85, 53, 33, and 24 samples belong to bahiagrass, bermudagrass, limpograss, and smutgrass, respectively. A comparison of the top mycotoxins across these grass species (Figure 4) shows that the three grass species, other than bahiagrass, had higher concentrations of at least two mycotoxins each. For example, relatively higher doses of zearalenone-4-sulfate were found in bermudagrass (1,850 ppb) and limpograss (1,160 ppb) compared to bahiagrass (89 ppb). Limpograss contained a higher dose of ochratoxin alpha (2,165 ppb) compared to other examined grass species. At least one top mycotoxin was shared among bahiagrass, bermudagrass, and limpograss. However, the top mycotoxins present in smutgrass weed were different compared to the other grass species. Smutgrass had higher doses of dihydrolysergol (1,178 ppb) and elymoclavine (1,499 ppb). Elymoclavine is the ergot alkaloid (a toxic compound that may cause serious health issues if ingested by animals) produced by *Claviceps* spp.(Bragg et al. 2017). Elymoclavine has been found to be present in plants belonging to the families Convolvulaceae, Poaceae, and Polygalaceae, as well as in grass- and cereal-based food and products (Krska and Crews 2008; Wallwey and Li 2011). It is important to clarify that the data does not mean bermudagrass and limpograss are harmful to livestock. As indicated in the previous section, there is currently no solid database to identify the threshold levels of many mycotoxins that could pose a risk to our livestock during consumption. More clinical data are needed to establish these thresholds. Nevertheless, this data report can still raise awareness among veterinarians and offer valuable insights for them to correlate observed symptoms in cattle grazing on these grass species.

## The Fluctuating Distribution of Mycotoxins Across Florida Ranches Depending on Seasons and Years

The 195 samples reported in this publication were collected from 13 Florida ranches, with 8, 168, and 19 samples collected during the dry/cooler season (October–April) and wet/warmer season (May–September) in 2017, 2018, and 2019, respectively. The results showed that the dominant mycotoxins present across all years and seasons were zearalenone-4-sulfate, emodin, and ochratoxin alpha (Figure 5). These data suggest that these three metabolites may be consistently present in the Florida pastures over time. However, the distribution of their concentrations over the years was inconsistent (Figure 5A). In 2017, ochratoxin alpha was the most prevalent, followed by zearalenone-4-sulfate and emodin. In contrast, the most dominant mycotoxin in 2018 and 2019 was zearalenone-4-sulfate followed by ochratoxin alpha and emodin. In addition, we observed different patterns of changes in the concentration of these three mycotoxins over the years. There was a "U" pattern for zearalenone-4-sulfate and ochratoxin alpha, with the highest concentration (1,410 ppb) of zearalenone-4-sulfate in 2019 and the highest concentration (1,500 ppb) of ochratoxin alpha in 2017. By contrast, emodin's concentration slightly increased from 2017 to 2018 before remaining stable until 2019. This information suggests that the concentrations of these three dominant metabolites can fluctuate annually.

When comparing the concentration of these three metabolites between the dry/cooler and wet/warmer seasons (Figure 5B), we found that the concentration of zearalenone-4-sulfate was substantially higher during the dry/cooler season, with levels being seven-fold and four-fold higher than emodin and ochratoxin alpha, respectively. In contrast, during the wet/warmer season, the concentration of these three dominant mycotoxins was relatively evenly distributed, ranging between 334 and 423 ppb.

Overall, annual monitoring would help predict the concentration of these mycotoxins, especially checking for zearalenone-4-sulfate during the dry season and ochratoxin alpha during the wet season.

## Management Recommendations

We hope the information reported here helps ranchers become aware of the mycotoxins in Florida's pastures and may encourage them to fine-tune their management practices to mitigate the potential impact of these toxins on animal health. This report is based on the data from 195 field samples collected over three years starting in 2017. More samples are needed to investigate in following years to refine the report outcomes. Ongoing data collection will continue to characterize the mycotoxin issue in Florida pastures. However, at this point, no management practices are recommended to prevent or mitigate mycotoxins in pastures or hay, including for animal feeding specifically in Florida (Liao et al. 2023).

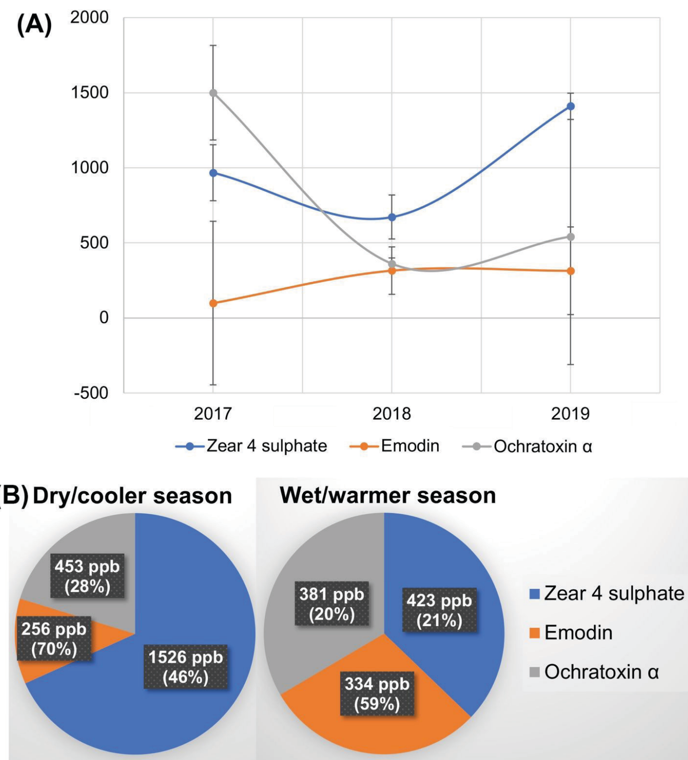


Figure 5. The dominant mycotoxins (top 3) in Florida's ranches from 2017 to 2019 (A) and in two seasons (B). (A) Error bars indicate standard errors of each variable. (B) Value and percentage in parentheses within each part of the pie graph represent the average concentration of each dominant mycotoxin and its prevalence.
Credits: Kaile Zhang, UF/IFAS

In addition to the broad survey findings, detailed observations at the ranch level can also provide valuable insights for management decisions. For example, our survey in Florida pastures revealed an uneven distribution of mycotoxins within and between pastures; some may exhibit high toxin levels while adjacent ones remain unaffected by the same toxins. Since mycotoxins are a significant concern in US pastures (e.g., fescue toxicosis), we outlined the following general recommendations that are also applicable to Florida pastures:

1. If veterinarians suspect clinical symptoms related to mycotoxins in cattle, the grazing area should be inspected before the next grazing period. If possible, remove the cattle from the affected area and allow them to graze in a nearby area with lower or no detectable toxins.

2. For pastures that consistently show high concentrations of certain mycotoxins, consider inter-seeding with legumes or other grasses to dilute the toxin levels.

3. Prevent the growth of smutgrass weed in pastures to avoid introducing additional types of mycotoxins.

## Summary/Highlight

1. The dominant mycotoxins and metabolites found in Florida's pastures were zearalenone-4-sulfate, ochratoxin, emodin, ochratoxin alpha, dihydrolysergol, and elymoclavine.

2. The concentrations of these dominant toxins can be associated with geographic locations, grass species, and Florida's seasons.

3. Zearalenone-4-sulfate was found as the dominant toxin in central Florida's ranches.

4. Among the three common grasses and one weed (smutgrass) examined in Florida, bahiagrass has relatively lower concentrations of mycotoxins. Relatively higher concentrations of zearalenone-4-sulfate were found in bermudagrass and limpograss. Smutgrass contains different types of mycotoxins, such as dihydrolysergol and elymoclavine.

5. Zearalenone-4-sulfate, emodin, and ochratoxin alpha were the top three metabolites constantly present in Florida's pastures across the seasons and years. However, the concentrations of these three metabolites can fluctuate annually, with potentially higher concentrations of zearalenone-4-sulfate in the dry/cooler season and higher levels of ochratoxin alpha in the wet/warmer season.

6. Zearalenone concentrations in 33.3% and 26.7% of the collected samples across Florida exceeded the maximum tolerable intake levels established by the JECFA (0.5 ppb) and Dairy One (10 ppb), respectively. This issue was especially pronounced in ranches in the central and south Florida regions, with significant instances at SR2 (65%), CR1 (50%), and CR6 (75%), in contrast to those in the north Florida region.

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Table 1. The prevalence and concentration (ppb) of dominant mycotoxins in collected grass leaves across Florida's ranches (years 2017–2019).

| Ranch (sample size) | Zearalenone | Alpha-zearalenol | Beta-zearalenol | Zear-4-sulphate | Emodin | Beauvericin | Dihydrolysergol | Alternariol | Alternariol Methyl ether | Ochratoxin α |
|---|---|---|---|---|---|---|---|---|---|---|
| | Minimum-median-maximum (prevalence; %) | | | | | | | | | |
| SR1 (5) | 0-0-0 (0) | 0-0-0 (0) | 0-0-0 (0) | 0-0-0 (0) | 16-26-798 (100%) | 0-0-7 (40%) | 0-0-67 (20%) | 0-0-59 (20%) | 0-0-35 (20%) | 0-0-0 (0) |
| SR2 (20) | 0-23-944 (65%) | 0-5-234 (45%) | 0-0-1276 (35%) | 0-334-9039 (65%) | 0-45-2722 (90%) | 0-4-135 (70%) | 0-0-0 (0) | 0-0-611 (30%) | 0-37-1034 (75%) | 0-0-826 (10%) |
| SR3 (24) | 0-0-372 (25%) | 0-0-265 (13%) | 0-0-149 (17%) | 0-0-5268 (25%) | 0-13-281 (58%) | 0-0-156 (33%) | 0-0-0 (0) | 0-0-179 (21%) | 0-9-189 (54%) | 0-0-4596 (29%) |
| CR1 (18) | 0-7-281 (50%) | 0-0-302 (22%) | 0-0-499 (33%) | 0-137-8024 (56%) | 0-12-227 (67%) | 0-0-437 (33%) | 0-0-0 (0) | 0-0-0 (0) | 0-0-24 (22%) | 0-0-3526 (39%) |
| CR2 (21) | 0-0-0 (0) | 0-0-1168 (33%) | 0-0-1431 (33%) | 0-0-16554 (38%) | 0-0-6230 (48%) | 0-0-1054 (38%) | 0-0-0 (0) | 0-0-258 (10%) | 0-0-457 (19%) | 0-0-1735 (38%) |
| CR3 (13) | 0-0-1545 (46%) | 0-0-294 (23%) | 0-0-491 (23%) | 0-0-9294 (38%) | 0-152-5202 (85%) | 0-0-66 (15%) | 0-0-0 (0) | 0-0-0 (0) | 0-0-33 (46%) | 0-0-4107 (31%) |
| CR4 (13) | 0-0-483 (38%) | 0-0-0 (0) | 0-0-0 (0) | 0-0-674 (23%) | 0-22-125 (69%) | 0-1-231 (62%) | 0-0-0 (0) | 0-0-149 (15%) | 0-10-203 (77%) | 0-0-6526 (46%) |
| CR5 (11) | 0-0-168 (36%) | 0-0-19 (9%) | 0-0-101 (9%) | 0-0-1052 (18%) | 0-14-2823 (64%) | 0-0-0 (0) | 0-0-763 (9%) | 0-0-230 (36%) | 0-0-123 (45%) | 0-0-0 (0) |
| CR6 (12) | 0-5-328 (75%) | 0-0-30 (17%) | 0-0-52 (8%) | 0-86-2321 (50%) | 0-11-1705 (58%) | 0-0-0 (0) | 0-0-0 (0) | 0-0-183 (33%) | 0-24-90 (100%) | 0-0-232 (8%) |
| NR1 (17) | 0-0-5 (6%) | 0-0-33 (12%) | 0-0-151 (6%) | 0-0-59 (6%) | 0-23-22652 (65%) | 0-0-245 (47%) | 0-0-0 (0) | 0-0-408 (29%) | 0-21-650 (71%) | 0-0-8415 (35%) |
| NR2 (19) | 0-0-126 (5%) | 0-0-0 (0) | 0-0-0 (0) | 0-0-111 (5%) | 0-7-293 (53%) | 0-0-32 (42%) | 0-0-13910 (26%) | 0-0-87 (16%) | 0-0-150 (47%) | 0-0-27 (5%) |
| NR3 (10) | 0-0-3 (10%) | 0-0-0 (0) | 0-0-0 (0) | 0-0-0 (0) | 0-1-82 (60%) | 0-0-25 (30%) | 0-0-68 (10%) | 0-13-393 (50%) | 0-41-253 (80%) | 0-0-0 (0) |
| NR4 (12) | 0-0-1 (8%) | 0-0-0 (0) | 0-0-0 (0) | 0-0-0 (0) | 0-0-0 (0) | 0-0-13 (42%) | 0-0-585 (25%) | 0-0-100 (333%) | 0-13-214 (100%) | 0-0-236 (8%) |

Note: Prevalence refers to the proportion or percentage of samples that contain mycotoxins.

Table 2. The percentage of the 195 grass samples containing mycotoxins above the maximum tolerable intake levels harmful to cattle as suggested by Dairy One, the JECFA, and the FDA (Food and Drug Administration).

| Mycotoxins | Percentage (%) of sample size higher than the tolerable intake levels as suggested by: | | |
|---|---|---|---|
| | Dairy One | JECFA | FDA |
| Zearalenone | 26.7% | 33.3% | NA |
| Ochratoxin A | 1.0% | 1.0% | NA |
| T-2 | 1.5% | NA | NA |
| Fumonisin B1 | 3.1% | 5.6% | 4.6% |
| Fumosinin B2 | 2.1% | NA | NA |

NA: The suggested maximum tolerable intake levels are not provided.