Chapter 10

Mycotoxins in DDGS

Introduction

Like all feed ingredients, distiller's grains may contain, at times, amounts of mycotoxins that can negatively affect animal performance, or be produced and stored under conditions that cause mold growth and mycotoxin production.

Mycotoxins can be present in DDGS if the grain delivered to an ethanol plant is contaminated with them. Mycotoxins are not destroyed during the ethanol production process, nor are they destroyed during the drying process to produce DDGS. In fact, if mycotoxins are present in corn, their concentration will be increased by a factor of about 3 times in DDGS. However, the risk of mycotoxin contamination in U.S. DDGS is very low because it is uncommon for most of the major corn growing regions in the U.S. to have climatic and weather conditions that lead to mycotoxin production in corn. Furthermore, most ethanol plants monitor grain quality and reject sources that are contaminated with mycotoxins.

Potential Mycotoxins in Corn and DDGS

Mycotoxins are secondary metabolites of fungi that can adversely affect the health, growth, or reproduction of animals, especially humans and domestic animals. Aflatoxins, including aflatoxin B1, B2, G1, and G2, are the most toxic and carcinogenic of the known mycotoxins, and they are produced by several *Aspergillus* species. Corn becomes susceptible to aflatoxin formation during under drought conditions during the growing season, or in high moisture or high humidity storage conditions (Richard, 2000).

Fusarium graminearum is the primary deoxynivalenol-producing fungus in grains in the United States (CAST, 2003). Deoxynivalenol (sometimes referred to as DON or vomitoxin) may coexist with other mycotoxins, such as zearalenone. *Fusarium graminearum* survives in old infested residue remaining in the field from the previous growing season, and cold, moist conditions are favorable for the fungus to grow on corn. Generally, storage is not considered a potential source for deoxynivalenol contamination if the corn was mature and stored at moisture level lower than 14% (Richard, 2000).

Fusarium verticillioides is the primary fungus capable of producing the fumonisins FB1, FB2, and FB3 (Gelderblom et al., 1988). Corn is the major grain commodity affected by this fungus. The specific conditions needed for the production of fumonisins are unknown, but it is suggested that drought stress followed by warm, wet weather during flowering seems to be important. *Fusarium verticillioides* is present in virtually every seed and is also present in the corn plant throughout its growth, and sometimes, there is a considerable amount of fumonisins present in symptomless kernels of corn. Since the discovery of this mycotoxin was fairly recent (1988), there is little information available regarding its production as well as its potential negative effects on animal health and performance (Richard, 2000).

Fusarium sporotrichioides is the principal fungus responsible for the production of T-2 toxin, which is a member of fungal metabolites known as the trichothecenes. The production of T-2 is the greatest under conditions of increased humidity and temperatures of 6–24 °C (CAST, 2003).

Zearalenone is an estrogenic fungal metabolite, and *Fusarium graminearum* is the major fungus responsible for producing this mycotoxin. Moist, cool growing conditions are favorable for this fungus to grow, and are the same conditions ideal for the production of deoxynivalenol. Maintaining moisture content of grain and grain co-products less than 14% is important to avoid zearalenone production.

Mycotoxin Testing

Since the 1960's, many analytical methods have been developed for the testing of mycotoxins in human food and animal feeds due to the concern of toxicity for human health (Trucksess, 2000). Among them, the methods of thin-layer-chromatography (TLC), enzyme-linked immunosorbent assay (ELISA) and immunosensor-based methods have been widely used for rapid screening, while high-performance liquid chromatography (HPLC) with fluorescence detection (FD) and mass spectrometry detection (MS) have been used as confirmatory and reference methods (Krska et al, 2008). However, due to the need for rapid, accurate and more economical on-site methods of mycotoxin determinations, testing kits approved for use on DDGS by the Grain Inspection, Packers and Stockyards Administration (GIPSA) of the United States Department of Agriculture are shown in **Table 1** (http://www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=lr&topic=hb).

Table 1. Mycotoxin Testing Kits for DDGS (Approved by GIPSA).

Brand Name	Manufacturer	Test Range	Test Format	Extraction	Clean-up
Aflatoxin					
Veratox Aflatoxin	Neogen Corporation	5–50 ppb	Microtiter Well Plate Assay	Methanol/water (70 + 30)	ELISA
Ridascreen FAST SC	R-Biopharm	5–100 ppb	Microtiter Well Plate Assay	Methanol/water (70 + 30)	ELISA
Aflatest	Vicam	5–100 ppb	Immunoaffinity Column	Methanol/water (80 + 20)	Affinity column
FluroQuant® Afla IAC	Romer	5–100 ppb	Fluorometry	Methanol/water (80 + 20)	Affinity column
Fumonisin					
AgraQuant Total Fumonisin 0.25/5.0	Romer	0.5–5 ppm	Direct Competitive ELISA	Methanol/water (70 + 30)	ELISA
Zearalenone					
ROSA® Zearalenone	Charm Sciences, Inc.	50–1000 ppb	Lateral Flow Strip	Methanol/water (70 + 30)	

Zhang et al., 2009

These methods are for detection of a single mycotoxin, allow for ease of operation, and are quantitatively sensitive allowing high sample throughput. There are six GIPSA approved methods for testing mycotoxins in DDGS (four methods for aflatoxin, one method for fumonisin, and one method for zearalenone).

When considering testing DDGS for mycotoxin contamination, it is essential to use approved analytical procedures to get accurate results. High performance liquid chromatography (HPLC) is the preferred method to determine the presence and level of mycotoxin in animal feeds. Using HPLC and a variety of detectors, most of the mycotoxins in animal feeds can be separated and detected (Krska et al, 2008). The methods used by major DDGS testing labs in the U.S. are described in **Table 2** and have been validated by individual labs and recently published in peer-reviewed scientific journals.

Table 2. Methods for mycotoxin testing in animal feed.

Target	Testing	Detection Range	Reference
Aflatoxin			
Corn, almonds, Brazil nuts, peanuts and pistachio nuts	HPLC – FD	5 – 30 ppb	AOAC 994.08
Deoxynivalenol			
Cereals and cereal products	HPLC – UV	.1 ppm (detection limit)	MacDonald et al., 2005a
Fumonisin			
Corn and corn flakes	HPLC – FD	0.5 – 2 ppm	AOAC 2001.04
Corn and corn-based feedstuffs	Thin layer chromatography (TLC)	.1 ppm (detection limit)	Rottinghaus et al., 1992
T-2			
Food and feed	Thin layer chromatography (TLC)	.1 ppm (detection limit)	Romer, 1986
Zearalenone			
Corn, wheat and feed	Microtiter Well Plate Assay	0.8 ppm (detection limit)	AOAC 994.01
Barley, maize and wheat flour, polenta, and maize-based baby foods	HPLC – FD	0.05 ppm (detection limit)	MacDonald et al., 2005b

Aflatoxins, Deoxynivalenol, Fumonisin, T-2, Zearalenone

Food and feed	LC/MS/MS	Aflatoxins (1 – 100 ppb); Deoxynivalenol, (1, 1000 ppb) Fumonisin (16 – 3,200 ppb) T-2, (2 – 1,000 ppb) Zearalenone (20 – 1,000 ppb)	Sulyok et al., 2007

Adapted from Zhang et al. (2009).

Maximum tolerable levels of mycotoxins in animal feed

The U.S. FDA has established maximum tolerable levels for aflatoxins (**Table 3**), deoxynivalenol (**Table 4**), and fumonisin (**Table 5**) in feed ingredients for various types of animal feeds. No action levels, advisory levels or guidance levels have been published by the FDA for T-2 toxin or zearalenone.

Table 3. FDA action levels for aflatoxin in complete feeds and feed ingredients.[1]

Animals	Action Levels (ppb)
Finishing beef (i.e., feedlot) cattle	300
Finishing swine (> 100 pounds)	200
Breeding beef cattle, breeding swine or mature poultry	100
Immature animals, dairy cattle or intended use unknown	20

[1] Zhang et al., 2009.

Table 4. FDA action levels for deoxynivalenol in complete feeds and feed ingredients.[1]

Animals	Advisory Levels (ppm)
Ruminating beef and feedlot cattle older than 4 months, and chickens with the added recommendation that these ingredients not exceed 50% of the diet	10
All other animals with the added recommendation that these ingredients not exceed 40% of the diet	5
Swine with the added recommendation that these ingredients not exceed 20% of the diet	5

[1] Zhang et al., 2009.

Table 5. FDA Action levels for fumonisin in complete feeds and feed ingredients.[1]

Animals	Recommended Guidance Levels (ppm)

Poultry raised for slaughter, no more than 50% of the diet	100
Ruminants older than 3 months raised for slaughter and mink being raised for pelt production, no more than 50% of the diet	60
Breeding ruminants, poultry, and mink, no more than 50% of the diet	30
Swine and catfish, no more than 50% of the diet	20
All other species or classes of livestock and pet animals, no more than 50% of the diet	10
Equids and rabbits, no more than 20% of the diet	5

1 Zhang et al., 2009.

Presence and Concentrations of Mycotoxins in U.S. DDGS

Zhang et al. (2009) conducted an extensive literature review of published studies and evaluated samples from three large data sets of DDGS samples to determine the extent and level of mycotoxin contamination among U.S. DDGS sources. Concentrations of all mycotoxins in DDGS were generally below the FDA action levels for all mycotoxins. There were only a couple of exceptions where the concentrations of deoxynivalenol or fumonisins were either at, or slightly above, the recommendations for selected sensitive animal species, and in those instances the occurrence rate was less than 10% of all samples tested, and these concentrations were below any harmful concentration when the DDGS would be added with other ingredients to make up the overall animal diet.

Caupert et al. (2011) published additional DDGS mycotoxin concentrations from multiple surveys and concluded that all concentrations of mycotoxins in DDGS were generally below the FDA regulations for the specific mycotoxins. In only a couple of instances where the concentrations of deoxynivalenol or fumonisins either at, or slightly above, the recommendations for sensitive animal species, and in those instances the occurrence was in less than 10% of the samples tested. These concentrations would be well below any harmful concentration when the DDGS is blended with other ingredients to make up the overall animal diet.

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FDA websites:

Aflatoxin in feeds and feed ingredients: http://www.cfsan.fda.gov/~lrd/fdaact.html#afla

Fumonisins in feeds and feed ingredients: http://www.cfsan.fda.gov/~dms/fumongu2.html

Deoxynivalenol (DON) in feeds and feed ingredients: http://www.cfsan.fda.gov/~dms/graingui.html

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