Scientific Overview of the Presence and Levels of Mycotoxins in U.S. DDGS

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Executive Summary

The motivation behind this paper was to draw scientifically sound conclusions on recent studies and publications that address the occurrence of mycotoxins in DDGS. Data from two independent studies, one research study and two publications were reviewed for this paper. It is difficult to draw scientifically sound conclusions from the two publications (Biomin and South Dakota State University), because those publications did not reveal the sampling source or sampling procedure for their studies. However, data provided from the two independent studies and the research study was sufficient to allow for a scientifically sound conclusion.

Data from the two independent studies and one research study contributed a total of 235 DDGS samples collected from 20 ethanol plants in the Midwestern United States and 23 export shipping containers. The time period in which samples were collected was from 2006 to 2008. The samples were tested for aflatoxin, deoxynivalenol, fumonisin, T-2 toxin and zearalenone using state-of-the-art analytical methodology. The results suggest:

- None of the samples contained aflatoxin or deoxynivalenol levels higher than FDA (U.S. Food and Drug Administration) guidelines for use in animal feed.
- No more than 10% of the samples contained fumonisin levels higher than the recommendation for feeding equids and rabbits, and the rest of the samples contained fumonisin lower than FDA guidelines for use in animal feed.
- No FDA guidance levels are available for T-2 toxin. None of the samples contained T-2 higher than the detection limit.
- No FDA guidance levels are available for zearalenone, and most samples contained zearalenone levels lower than detection limit.
- The containers used for export shipping of DDGS do not seem to contribute to mycotoxin production.

Well-enforced sampling and sample preparation are crucial for accurate results in mycotoxin testing. Rapid and cost-effective, on-site testing kits for DDGS have been approved by the USDA (United States Department of Agriculture), and highly sensitive instrumental methods for analysis of mycotoxins in DDGS are also available in the market. The fact that mycotoxins are detectable has no relationship with their toxicity in any animal species.

Introduction

Mycotoxins are unavoidable contaminants in crops (CAST, 2003) and therefore they also occur in commodities entering the marketing chain including those grains to be used in ethanol production. Currently, corn (maize) is the primary commodity used for the production of ethanol in the U.S. However, depending on geographical location of an ethanol plant and price relative to corn, sorghum and wheat are sometimes used, or blended with corn to produce ethanol and DDGS. Several mycotoxins can potentially be found in corn including aflatoxin, deoxynivalenol (DON), fumonisin, T-2 toxin and zearalenone (ZON). Most of these toxins can occur in corn,

pre-harvest, and become present in the grain at harvest, however, such occurrence is dependent upon the unique environmental conditions that are conducive to the growth of specific molds that produce these mycotoxins during crop development. Therefore, mycotoxin contamination in corn is not an annual event because the appropriate environmental conditions are often lacking for the growth of the specific responsible fungi. Among the toxins, T-2 toxin is not a major pre-harvest contaminant in grains and is likely a result of inadequate storage of grains allowing for their production by the responsible fungi occurring in the stored grain.

During the corn-to-ethanol production process, approximately two-thirds of the grain, mainly starch, is fermented by yeast to produce ethanol and carbon dioxide, neither of which would contain mycotoxins if contaminated corn was used. However, the remaining co-product, distiller's dried grains with solubles (DDGS), could potentially contain a higher concentration of any mycotoxin that was present in the grain prior to fermentation. The increased level of a given mycotoxin in DDGS was reported to be approximately three times as high as the level in the grain (Bennett & Richard, 1996; Bothast et al, 1992). The tremendous growth in the fuel-ethanol industry has been accompanied by concomitant growth in the production of DDGS, and the potential for increased use of DDGS as animal feed is great. As a result, more attention has been paid to the prevalence and levels of mycotoxins in DDGS.

In this report, we will discuss the state-of-the-art test methods available on the market for mycotoxin analysis in DDGS, and review several independent studies and publications that have been conducted for the determination of mycotoxins in DDGS. More importantly, we will compare the detectable mycotoxin levels in DDGS from those studies with current action levels, advisory levels and guidance levels of the FDA. This report is an attempt to consolidate the data from these studies and publications (data in all tables came directly from analytical laboratory reports or publications), and evaluate the meaning of the results relative to the potential for toxicity in animals to which the DDGS might likely be fed.

Regulations and Guidance

As of July, 2008, the regulatory levels of mycotoxins established by the FDA are for the use of feed ingredients as animal feed. The following discussion focuses on regulations or guidance for mycotoxins in feed ingredients from the FDA.

Aflatoxin

Action levels for aflatoxin in animal feed have been established for different animals and at different production stages. The FDA action level represents the minimum limit at which the FDA can take legal action to remove feed ingredients from the market. The following action levels were established in August, 2000.

Table 1a. FDA Action Levels for Aflatoxin in Feed Ingredients

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 is not known	20

Deoxynivalenol

The advisory levels for deoxynivalenol in animal feeds are set by the FDA as follows:

Table 1b. FDA Action Levels for Deoxynivalenol in Feed Ingredients

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 of cattle and chickens	10
All other animals with the added recommendation that these ingredients not exceed 40% of the diet of cattle and chickens	5
Swine with the added recommendation that these ingredients not exceed 20% of the diet of cattle and chickens	5

Fumonisin

According to guidance levels set by the FDA in 2001, the recommended maximum levels for fumonisin in animal feeds are as follows:

Table 1c. FDA Action Levels for Fumonisin in Feed Ingredients

Animals	Recommended Guidance Levels (ppm)
Poultry being 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

No action levels, advisory levels or guidance levels for T-2 toxin or zearalenone are available from the FDA.

Analytical Testing Methods for Mycotoxins

The testing for mycotoxins in DDGS involves obtaining an adequate sample, preparing the sample for the analytical test, and choosing a quality method for the specific mycotoxins of interest. Every step of the process is important in order to obtain results that accurately reflect the mycotoxin concentration in the original lot (CAST, 2003).

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). In this paper, we will discuss the state-of-the-art in the analysis of mycotoxins in DDGS by focusing on testing kits which have been approved by the Grain Inspection, Packers and Stockyards Administration (GIPSA) of the United States Department of Agriculture (USDA GIPSA website), and the confirmatory methods which are used by major DDGS testing labs in the U.S. (Zhang and Sido, 2008).

Sample Collection

The greatest variability or source of error in overall mycotoxin testing comes from sampling (CAST, 2003). To obtain accurate mycotoxin results, it is crucial to establish a well-enforced sampling program, which ensures enough number of sampling probes, sufficient sample size and appropriate particle size. Once an adequate sample is collected, it must be ground to reduce particle size, mixed thoroughly and then an aliquot is taken for analysis.

The goal of sampling is to remove an appropriate quantity for testing from a large bulk lot, in such a way that the proportion and distribution of the factors being tested are the same in both the whole (lot) and the part removed (sample) (Richard, 2006). The sampling process consists of taking a number of small samples from a lot and pooling them into a large composite sample. For a sample to be considered representative, it must be:

1. Obtained with appropriate equipment, such as probe (trier) for stationary grain, a diverter-type mechanical sampler or pelican sampler for moving grain.
2. Obtained using a sampling pattern and procedure designed to collect samples from all areas of the lot to make a composite sample.
3. Of appropriate size. The GIPSA recommended minimum sample size for an aflatoxin test is two pounds (about 1 kg) from a truck load, three pounds (about 1.5 kg) from a railcar load, and 10 pounds (about 4.5 kg) from a barge load.
4. Adequately identified and labeled.
5. Handled in appropriate way, such as stored in cool and dry place, placed in double or triple lined paper bags or breathable cloth bags, etc.

The detailed information on sampler and sampling pattern is listed at the USDA website (http://www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=lr&topic=hb).

Sample Preparation

One of the objectives of sample preparation is to obtain a small sample of grain to be used for analytical testing. Based on the recommendations from the Association of Analytical Chemists (AOAC) and the GIPSA, we suggest the following steps for sample preparation:

1. Grind the total sample using an appropriate mill to pass #14 mesh sieve. There are several grinders recommended by the GIPSA (USDA website).
2. Split the sample using a sample splitter until 1-2 kg is obtained.
3. Regrind 1-2 kg to completely pass a #20 mesh sieve.
4. Mix reground portion thoroughly in a tumble blender or planetary mixer.
5. Take a 500-gram, sub-sample from the mix for any analytical testing.

Sample Analysis

The determination of mycotoxins in DDGS can be divided into four steps: extraction of the sample, clean-up of the extract, separation and detection.

Mycotoxins are extracted from the sample to liberate the mycotoxins of interest from the sample matrix. The closer the recovery is to 100%, the more accurate the final analytical result will be. The most common extraction solution used is a mixture of water and other polar solvents.

Cleanup of extracts is usually necessary to eliminate interfering substances from the sample matrix to ensure better selectivity of the detection and allow for any low level of mycotoxins from the original sample to be detected. The current clean-up technology usually employs immunoaffinity columns (IACs) or multifunctional MycoSep columns (Krska et al, 2008).

There has been a high demand for rapid and cost-effective, on-site determination of mycotoxins. These methods usually are for detection of a single mycotoxin, allow for ease of operation, and sensitive quantitation with high sample throughput. As of September, 2008, there are six GIPSA approved methods for testing mycotoxins in DDGS; four of them for aflatoxin, one for fumonisin and one for zearalenone (USDA GIPSA website; Table 2).

Table 2. 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)	

High performance liquid chromatography (HPLC) has become the method of choice for confirmatory analysis of mycotoxin levels in animal feeds. Coupled with a variety of detectors, most of the mycotoxins in animal feeds are capable of being separated and detected by HPLC (Krska et al, 2008). The methods used by major DDGS testing labs in the U.S. are described in Table 3. These methods have been validated by individual labs and recently published in peer-reviewed scientific journals.

Liquid chromatography with mass spectrometry detection (LC/MS) has gained considerable attention recently because this technology can simultaneously detect and identify multi-mycotoxins in animal feed. This method provides definite confirmation of the molecular identity, uses simple extraction with no clean-up, and has high selectivity and sensitivity (Table 3).

Table 3. Instrumental Methods for Mycotoxin Testing in Animal Feed

Target	Testing	Detection Range	Extraction	Cleanup	Reference
Aflatoxin					
Corn, almonds, Brazil nuts, peanuts and pistachio nuts	HPLC – FD	5 – 30 ppb	Acetonitrile - water (90 + 10)	MycoSep column	AOAC 994.08
Deoxynivalenol					
Cereals and cereal products	HPLC – UV	0.1 ppm (detection limit)	Water	Immunoaffinity column	MacDonald et al., 2005a
Fumonisin					
Corn and corn flakes	HPLC – FD	0.5 – 2 ppm	Methanol - acetonitrile - water (25 + 25 + 50)	Immunoaffinity column	AOAC 2001.04
Corn and corn-based feedstuffs	Thin layer chromatography (TLC)	0.1 ppm (detection limit)	Acetonitrile - water (50 + 50)	C-18 column	Rottinghaus et al., 1992
T-2					
Food and feed	Thin layer chromatography (TLC)	0.1 ppm (detection limit)	Acetonitrile - water (84 + 16)	Charcoal / alumina	Romer, 1986
Zearalenone					
Corn, wheat and feed	Microtiter Well Plate Assay	0.8 ppm (detection limit)	Methanol – water (70 + 30)	MycoSep column	AOAC 994.01
Barley, maize and wheat flour, polenta, and maize-based baby foods	HPLC – FD	0.05 ppm (detection limit)	Acetonitrile – water	Immunoaffinity column	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)	Acetonitrile - water – acetic acid (79 + 20 + 1)		Sulyok et al., 2007

Review and Analysis of Independent Data

The following review is based on data from two independent studies:

1. A study conducted by the National Corn-to-Ethanol Research Center (NCERC) which included 20 DDGS samples collected from 14 ethanol plants from seven states in the Midwest U.S. from 2007 to 2008.
2. A larger data set provided by a Midwestern U.S. ethanol producer which included 162 samples collected from four ethanol plants from 2006 to 2008.

The results from those studies provide a reasonable representation of the occurrence and levels of major mycotoxins in DDGS in the U.S. during this time period.

1. Study Conducted by the National Corn-to-Ethanol Research Center (NCERC) (2008)

Sampling and testing methods

The NCERC conducted a study of mycotoxin prevalence and levels in DDGS based on samples from their National DDGS Library (Zhang et al, 2008). The 20 samples from the Library were collected from 14 ethanol plants representing seven states in the Midwestern United States (six ethanol plants located in five different states sent samples twice within a nine-month period). Samples were collected at the ethanol plants immediately after they were produced and shipped to the NCERC overnight. Immediately after they arrived, the samples were vacuum sealed and stored in a freezer at -20^{o} C.

The mycotoxin tests were performed at a commercial testing lab. Samples were analyzed for aflatoxin B_1, B_2, G_1, G_2, deoxynivalenol, fumonisin B_1, B_2 and B_3, and zearalenone by HPLC methodology and for T-2 toxin by TLC. The detection limits for the tests were 1 ppb for each aflatoxin, 0.1 ppm for deoxynivalenol, 0.1ppm for each fumonisin, 0.1ppm for T-2 toxin, and 0.05 ppm for zearalenone.

Results

Almost none of the 20 samples contained mycotoxin levels higher than the recommendation levels established by the FDA for animal feeds. The exceptions were two DDGS samples that contained fumonisin higher than 5 ppm, but lower than 10 ppm. The results of this study can be found in Table 4.

Aflatoxin:

- 70% of the samples were below the limit of detection of 1 ppb, thus they can be described as being Non-Detect.
- The maximum level detected was 3.7 ppb, which is well below all FDA action levels.
- The average level of aflatoxin across all 20 samples was 0.7 ppb.
- **Conclusion:** All 20 samples were below the lowest action level established by the FDA for all animal species.

Deoxynivalenol:

- 25% of the samples were below the limit of detection of 0.1 ppm, thus they can be described as being Non-Detect.
- The maximum level detected was 1.2 ppm, which is lower than all FDA advisory levels.
- The average level of deoxynivalenol across all 20 samples was 0.3 ppm.
- **Conclusion:** All 20 samples were below the lowest advisory level established by the FDA for all animal species.

Fumonisin:

- Only two DDGS samples contained total fumonisin levels in excess of the lowest recommended guidance levels established by the FDA.
- The average level of fumonisin across all 20 samples was 1.9 ppm.

- **Conclusion**: The average concentration of fumonisin, across all 20 samples, was below the lowest recommended guidance levels established by the FDA for all animal species.

T-2 toxin:

- There are no action levels, advisory levels or guidance levels for T-2 by the FDA.
- None of the 20 samples had a level of T-2 that exceeded the limit of detection of 0.1 ppm.
- **Conclusion:** All 20 samples can be described as being Non-Detect for T-2.

Zearalenone:

- There are no action levels, advisory levels or guidance levels for zearalenone by the FDA.
- 55% of the samples were below the detection limit of 0.05 ppm.
- The average concentration of zearalenone across all 20 samples was 0.038 ppm.
- **Conclusion:** The average concentration of zearalenone across all 20 samples was below the detection limit of 0.05 ppm.

These results suggest that aflatoxin, deoxynivalenol, zearalenone, and fumonisin, but not T-2 toxin, were detected in these samples from a large geographic region in the U.S. (7 states). However, if a sample had detectable mycotoxins, they were well below the maximum tolerable guidelines for use in animal feeds with the exception of the two samples with fumonisin levels which could be a concern if these DDGS sources were fed to equids or rabbits, but not other animal species.

Table 4. Results of Mycotoxin Concentrations in DDGS (NCERC, 2008)

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	20	< 1	3.7	0.7	0 %
Deoxynivalenol (ppm)	20	< 0.1	1.2	0.3	0 %
Fumonisin (ppm)	20	< 0.1	8.6	1.9	10 %
T-2 toxin (ppm)	20	< 0.1	< 0.1	0	N.A.
Zearalenone (ppm)	20	< 0.05	0.143	0.038	N.A.

2. Study Conducted by an Ethanol Company with Multi Ethanol Plants (2006 – 2008)

Sampling and testing methods

From February of 2006 through November of 2007, DDGS samples were collected from two ethanol plants (Plant 1 in Table 5a; Plant 2 in Table 5b) of a Midwestern U.S. ethanol company for mycotoxin tests. More than one DDGS sample was collected on a monthly basis from each ethanol plant and sent to a commercial lab for mycotoxin testing. Between February of 2008 and July of 2008, DDGS samples from four ethanol plants (four plants combined in Table 5c) were collected weekly and sent to a commercial lab for mycotoxin testing.

Samples were analyzed for aflatoxin B_1, B_2, G_1, G_2, deoxynivalenol, fumonisin B_1, B_2 and B_3, T-2 toxin, and zearalenone. The methodology utilized by the first commercial lab is LC/MS. Detection limits for the tests were 1 ppb for each aflatoxin, 0.1 ppm for deoxynivalenol, fumonisins and T-2, and 0.05 ppm for zearalenone. The methodology utilized by the second commercial lab is HPLC. Detection limits for the tests were 3 ppb for aflatoxin B_1, 1 ppb for aflatoxin B_2, 15 ppb for aflatoxin G_1, 5 ppb for aflatoxin G_2, and 0.2 ppm for deoxynivalenol, fumonisin and zearalenone. T-2 was not tested at the second lab.

Results

Almost none of the 162 samples contained mycotoxins higher than the recommendation levels established by the FDA for animal feeds. Only two DDGS samples from Plant 1 and eight DDGS samples from the four combined plants contained fumonisin higher than 5 ppm, but they were all lower than 10 ppm. The results of this study can be found in Tables 5a, 5b and 5c.

Aflatoxin:

- 96% of the samples from Plant 1, 88% of the samples from Plant 2, and 99% of the samples from the four plants combined were below the limit of detection of 1 ppb, thus they can be described as being Non-Detect.
- The maximum level of aflatoxin detected for Plant 1 was 2.56 ppb, for Plant 2 was 1.21 ppb, and for the four plants combined was 1.12 ppb. These levels fall well below all FDA action levels.
- **Conclusion:** All 162 samples were below the lowest action level established by the FDA for all animal species.

Deoxynivalenol:

- The maximum level of deoxynivalenol detected in a sample from Plant 1 was 1.42 ppm, for Plant 2 was 1.68 ppm and for the four plants combined was 1.9 ppm. These levels fall below all FDA advisory levels.
- The average level of deoxynivalenol detected in the samples from Plant 1 was 0.64 ppm, for Plant 2 was 1.02 ppm and for the four plants combined was 0.5 ppm.
- **Conclusion:** All 162 samples were below the lowest advisory level established by the FDA for all animal species.

Fumonisin:

- The maximum level of fumonisin detected in a sample from Plant 1 was 5.88 ppm, for Plant 2 was 2.77 ppm and for the four plants combined was 7.2 ppm.
- The average level of fumonisin detected in the samples from Plant 1 was 2.33 ppm, for Plant 2 was 1.47 ppm and for the four plants combined was 2.7 ppm.
- **Conclusion:** While 10 samples did have fumonisin levels above 5 ppm, those ten samples were all less than 10 ppm. The average concentration of fumonisin across all 162 samples was below the lowest recommended guidance levels established by the FDA for all animal species.

T-2 toxin:

- There are no action levels, advisory levels or guidance levels for T-2 by the FDA.
- None of the 69 samples submitted by Plant 1 or the 16 samples submitted by Plant 2 contained a concentration of T-2 above the 0.1 ppm level of detection. The 77 samples submitted by the four combined plants were not tested for T-2.
- **Conclusion:** All 85 samples that were tested for T-2 can be described as being Non-Detect.

Zearalenone:

- There are no action levels, advisory levels or guidance levels for zearalenone by the FDA.
- 68% of the samples from Plant 1 and 44% of the samples from Plant 2 fell below the limit of detection of 0.05 ppm. 100% of the samples from the four combined plants fell below 0.2 ppm.
- The maximum level of zearalenone for samples submitted from Plant 1 was 0.123 ppm, for Plant 2 was 0.113 ppm and for the four combined plants was <0.2 ppm.
- **Conclusion:** The average concentration of zearalenone across all 162 samples was below the 0.2 ppm detection limit.

Table 5a. Results of Mycotoxin Concentrations in DDGS (Plant 1, Lab 1, 2/2006 – 11/2007)

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	69	< 1	2.56	0.08	0 %
Deoxynivalenol (ppm)	69	< 0.1	1.42	0.64	0 %
Fumonisin (ppm)	69	0.12	5.88	2.33	3 %
T-2 toxin (ppm)	69	< 0.1	< 0.1	0	N.A.
Zearalenone (ppm)	69	< 0.05	0.123	0.025	N.A.

Table 5b. Results of Mycotoxin Concentrations in DDGS (Plant 2, Lab 1, 7/2006 – 11/2007)

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	16	< 1	1.21	0.15	0 %
Deoxynivalenol (ppm)	16	0.13	1.68	1.02	0 %
Fumonisin (ppm)	16	0.28	2.77	1.47	0 %
T-2 toxin (ppm)	16	< 0.1	< 0.1	0	N.A.
Zearalenone (ppm)	16	< 0.05	0.113	0.042	N.A.

Table 5c. Results of Mycotoxin Concentrations in DDGS (Four Plants Combined, Lab 2, 2/2008 - 7/2008)

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	77	< 1	1.12	0.01	0 %
Deoxynivalenol (ppm)	77	0.2	1.9	0.5	0 %
Fumonisin (ppm)	77	< 0.2	7.2	2.7	10 %
T-2 toxin (ppm)	Not Available	Not Available	Not Available	Not Available	N.A.
Zearalenone (ppm)	77	< 0.2	< 0.2	0	N.A.

Similar to the results from the NCERC (2008) study, these results suggest that aflatoxin, deoxynivalenol, fumonisin and zearalenone, but not T-2 toxin, were detected in a portion of the samples, but at very low levels, over a two-year time period. The levels detected in the majority of the samples were well below the maximum tolerable guidelines by the FDA for use in animal feed.

Review of Recent Study and Publications

Study Conducted by Iowa State University, Veterinary Diagnostic Lab and Novecta LLC (2006 & 2007)

In the Asian Pacific market there is a concern about the time, environment and shipping procedures of DDGS from the United States to foreign markets. The concern is that these factors support or enhance mold growth of the DDGS product. This study investigated the mycotoxin content in DDGS before and after shipment from a port in the United States to a port in Taiwan.

The project was conducted in two phases:

- Phase I was conducted in the Taiwan winter season of 2006. The study included 7 DDGS samples coming directly from different ethanol plants and 11 samples coming from U.S. port shipping containers resulting from those ethanol plants. All samples were acquired from different sources in the Midwestern United States, including Iowa, Illinois, Wisconsin and Minnesota. Samples were collected over a period of 3 months. The same 11 containers were sampled again upon arriving in Taiwan. The samples were then shipped back to the Veterinary Diagnostic Lab at Iowa State University for analysis.

- Phase II was conducted in the Taiwan summer season of 2007. The study included samples from 12 shipping containers at a U.S. port resulting from ethanol plants. Again the 12 shipping containers were sampled after arriving in Taiwan (summer season). The samples were then shipped back to the Veterinary Diagnostic Lab at Iowa State University for analysis.

Sampling and testing methods

Sampling of the shipping containers was to be done using the Kansas State University probe technique (Herrman, 2001). However, due to safety concerns, sampling was performed using pelican style sampling at the loading area taking 10 samples from the stream at varying intervals. This sample was mixed well and sub-sampled into 400- 500 gram samples before shipment. All sampling in the United States was overseen by USDA officials. Sampling in Taiwan, performed the same as in the United States, was overseen and performed by an independent sampler at the loading port in Taiwan. Samples from the shipping containers were assigned the container number for either U.S. or Taiwan origin so comparison of data could be drawn.

All analyses were performed at the Veterinary Diagnostic Lab at Iowa State University, Ames, Iowa. Samples received at the lab were stored at – 20°C until analysis could be performed. The samples were extracted using acetonitrile/water and cleaned up using solid phase extraction columns. The sample extract was screened for aflatoxin (B_1, B_2, G_1, G_2), deoxynivalenol, total fumonisin, T-2 and zearalenone/zearalenol by TLC. The detection limits of the TLC method were 5 ppb for each aflatoxin, 0.5 ppm for deoxynivalenol, fumonisin and zearalenone, and 1 ppm for T-2. For the samples with mycotoxin levels below the detection limit, every fifth sample extract was spiked with that specific mycotoxin and screened by TLC again to confirm test sensitivity. The spiking levels were 10 ppb for aflatoxin, 1 ppm for deoxynivalenol, fumonisin and zearalenone/zearalenol, and 2 ppm for T-2. For the samples with mycotoxin levels above the detection limit, a confirmatory test was performed using HPLC or GC (Stahr, 1991). The detection limits for the HPLC method were 0.5 ppb for each aflatoxin, 0.1 ppm for fumonisin and zearalenone, and the detection limits for GC method were 0.1 ppm for deoxynivalenol and 0.3 ppm for T-2.

Results

None of the 53 samples, from either Phase I or II, contained mycotoxin levels higher than the recommendation levels established by the FDA for animal feed. The results of this study can be found in Tables 6a through 6e.

Aflatoxin:

- None of the 53 samples had a level of aflatoxin that exceeded the limit of detection of 5 ppb.
- **Conclusion:** All 53 samples were below the lowest action level established by the FDA for all animal species.

Deoxynivalenol:

- Only four samples from Phase I, two from the ethanol plants and two from the Taiwan port, contained detectable deoxynivalenol levels. The maximum level detected was 3.4 ppm, which was lower than all FDA advisory levels (Table 6a).
- None of the samples from Phase II had a level of deoxynivalenol that exceeded the limit of detection of 0.5 ppm (Tables 6d and 6e).
- **Conclusion:** All 53 samples were below the lowest advisory level established by the FDA for all animal species. No increase in deoxynivalenol was observed in the shipment of DDGS from the U.S. to Taiwan.

Fumonisin:

- All 53 samples contained detectable fumonisin, but they were below the lowest recommended guidance levels established by the FDA for all animal species.
- The maximum level of fumonisin detected in a sample from Phase I was 2.9 ppm (Table 6a), and from Phase II was 2.4 ppm (Table 6e).
- In Phase I study, the average levels of fumonisin were found to be 2.3 ppm for the samples from the ethanol plants (Table 6a), 1.9 ppm for the samples from the U.S. port (Table 6b), and 1.2 ppm for the samples from the Taiwan port (Table 6c). In Phase II study, the average levels of fumonisin were found to be 0.9 ppm for the samples from the U.S. port (Table 6d) and 1.5 ppm for the samples from the Taiwan port (Table 6e).
- **Conclusion:** All 53 samples contained fumonisin lower than the lowest recommended guidance levels established by the FDA for all animal species. No increase in fumonisin was observed in the shipment of DDGS from the U.S. to Taiwan.

T-2 toxin:

- There are no action levels, advisory levels or guidance levels for T-2 by the FDA.
- None of the 53 samples had a level of T-2 that exceeded the limit of detection of 1 ppm.
- **Conclusion:** All 53 samples can be described as being Non-Detect for T-2.

Zearalenone:

- There are no action levels, advisory levels or guidance levels for zearalenone by the FDA.
- None of the 53 samples had a level of zearalenone that exceeded the limit of detection of 0.5 ppm.
- **Conclusion:** All 53 samples can be described as being Non-Detect for zearalenone.

This study suggested that only deoxynivalenol and fumonisin were detected in a portion of the samples, but at very low levels which were well below the maximum tolerable guidelines for use in animal feed. No apparent increase in mycotoxins was observed in the shipment from the U.S. to Taiwan during winter and summer seasons, which indicated that the port containers themselves did not contribute to mycotoxin production.

Table 6a. DDGS Samples Directly from Ethanol Plants

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	7	< 5	< 5	0	0 %
Deoxynivalenol (ppm)	7	< 0.1	3.4	0.6	0 %
Fumonisin (ppm)	7	1.8	2.9	2.3	0 %
T-2 toxin (ppm)	7	< 1	< 1	0	N.A.
Zearalenone/ Zearalenol(ppm)	7	< 0.5	< 0.5	0	N.A.

Table 6b. Phase I (winter season) DDGS Samples from U.S. Port Containers

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	11	< 5	< 5	0	0 %
Deoxynivalenol (ppm)	11	< 0.5	< 0.5	0	0 %
Fumonisin (ppm)	11	0.7	2.4	1.9	0 %
T-2 toxin (ppm)	11	< 1	<1	0	N.A.
Zearalenone/ Zearalenol(ppm)	11	< 0.5	< 0.5	0	N.A.

Table 6c. Phase I (winter season) DDGS Samples from Taiwan Port Containers

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	11	< 5	< 5	0	0 %
Deoxynivalenol (ppm)	11	< 0.1	1.0	0.1	0 %
Fumonisin (ppm)	11	0.7	2.0	1.2	0 %
T-2 toxin (ppm)	11	< 1	<1	0	N.A.
Zearalenone/ Zearalenol(ppm)	11	< 0.5	< 0.5	0	N.A.

Table 6d. Phase II (summer season) DDGS Samples from U.S. Port Containers

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	12	< 5	< 5	0	0 %
Deoxynivalenol (ppm)	12	< 0.5	< 0.5	0	0 %
Fumonisin (ppm)	12	0.5	1.4	0.9	0 %
T-2 toxin (ppm)	12	< 1	< 1	0	N.A.
Zearalenone/ Zearalenol(ppm)	12	< 0.5	< 0.5	0	N.A.

Table 6e. Phase II (summer season) DDGS Samples from Taiwan Port Containers.

Mycotoxins	Number of Samples Submitted	Minimum Level	Maximum Level	Average Level (of all samples)	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	12	< 5	< 5	0	0 %
Deoxynivalenol (ppm)	12	< 0.5	< 0.5	0	0 %
Fumonisin (ppm)	12	0.4	2.4	1.5	0 %
T-2 toxin (ppm)	12	< 1	< 1	0	N.A.
Zearalenone/ Zearalenol(ppm)	12	< 0.5	< 0.5	0	N.A.

"Crucial to monitor mycotoxins in DDGS" by Rodrigues, *Asian Feed* (2008)

Sampling and testing methods

There were 103 DDGS samples included in this study, among which 67% were from the United States and 33% were from Asia (Table 7). No information was provided on how representative the DDGS samples from the U.S. were, or how the DDGS samples were collected.

In this study, aflatoxin, fumonisin and zearalenone were analyzed by HPLC and deoxynivalenol was analyzed by TLC. The analytical method for measuring T-2 toxin was not described. The detection limits for aflatoxin, deoxynivalenol, fumonisin, T-2 and zearalenone were 0.5 ppb, 0.15 ppm, 0.025 ppm, 0.03 ppm and 0.01 ppm, respectively. The analyses were conducted at two different commercial laboratories, but it is unclear which samples were analyzed by which laboratories. It is well documented that considerable lab-to-lab variation can exist when analyzing various nutrients and toxins present in feedstuffs.

Results

This author reported that 99% of the samples contained at least one detectable mycotoxin, with 8% containing detectable aflatoxin, 64% containing detectable deoxynivalenol, 87% containing detectable fumonisin, 26% containing T-2 toxin and 92% containing detectable zearalenone. However, since all values for the mycotoxins were reported as maximums and averages, it was difficult to determine the percentage of these samples that contained concentrations of mycotoxins that were above the FDA action levels or recommended maximum tolerable levels for use in animal feed. Furthermore, it was not clear how many of the samples with detectable levels of mycotoxins were from DDGS produced in the U.S.

Aflatoxin: Only 8% of the DDGS samples contained aflatoxin levels higher than 0.5 ppb. The average (24 ppb) and maximum (89 ppb) concentrations found were below the FDA action levels for all animal species except for lactating dairy cattle, where the maximum tolerable level is 20 ppb. The proportion of the 8% DDGS samples containing aflatoxin levels higher than 20 ppb that were produced in Asia compared to the U.S. is unknown.

Deoxynivalenol: Approximately 64% of the DDGS samples contained deoxynivalenol levels higher than 0.15 ppm. However, the average concentration of deoxynivalenol detected in these DDGS samples was approximately 2 ppm, indicating that a high proportion of these samples could be used for all animal species. The maximum concentration detected among these samples was 12 ppm. This level is greater than the FDA advisory level for any animal species. It is unknown what proportion of the 64% of the DDGS samples contained deoxynivalenol levels higher than 5 ppm, or how many of those samples were produced in Asia compared to the U.S.

Fumonisin: A high proportion (87%) of the DDGS samples contained fumonisins higher than 0.025 ppm. The average concentration of fumonisins in the DDGS samples was around 0.6 ppm, and would be considered safe for use in feed for all animal species. The maximum concentration of fumonisins was 9 ppm which would be acceptable for use in feed for any animal species except for equids and rabbits. It is unknown what proportion of the 87% DDGS samples contained fumonisin concentrations greater than 5 ppm, or the number of those samples that were from Asia compared to the U.S.

T-2 toxin: Only 26% of the DDGS samples contained T-2 levels higher than 0.03 ppm. It would be interesting to know if any of the U.S. DDGS samples analyzed in this study contained this mycotoxin.

Zearalenone: Is not a regulated mycotoxin by the FDA and no recommendations have been formulated for industry guidance relative to incorporation of this mycotoxin in animal diets. Of the DDGS samples collected and analyzed in this study, 92% of them contained zearalenone concentrations higher than 0.01 ppm. It is unknown how many of the 92% DDGS samples contained zearalenone higher than 1 ppm, or the origin of those samples.

Table 7. Results of Mycotoxin Concentrations in DDGS (Rodrigues, 2008)

Mycotoxins	Number of Samples Submitted	Mean Conc. (of detectable levels)	Maximum Level	Percentage of Samples Above the Lowest FDA Level
Aflatoxin (ppb)	103	24	89	**N.A.***
Deoxynivalenol (ppm)	103	2.13	12.0	**N.A.**
Fumonisin (ppm)	103	0.596	9.042	**N.A.***
T-2 toxin (ppm)	103	0.113	0.218	**N.A.**
Zearalenone (ppm)	103	0.333	8.107	**N.A.**

* Data not available

The results of this study are difficult to interpret because the sampling methodology and geographic distribution of DDGS sources were not adequately described. Furthermore, the distribution of mycotoxin concentrations in contaminated samples was not reported. In addition, the author did not indicate what aflatoxins or fumonisins they measured. Although the maximum values for many of the mycotoxins were higher than those reported in previous U.S. studies, it appears that most of the samples contained levels below accepted FDA action levels.

"Mycotoxins in corn distillers grains, a concern for ruminants?" Garcia et al., South Dakota Cooperative Extension Service Extension Extra, March 2008: 1-3. (2008)

Sampling and testing methods

This study was a review of data generated by Dairy One Forage Laboratory in Ithaca, New York. The data were based on samples of DDGS and wet distiller's grains (WDG) submitted to the lab from 2000 through 2007 for mycotoxin analysis. No information was provided regarding the geographic distribution of the samples or how the DDGS samples were collected (Dairy One Forage Laboratory website).

Neogen Veratox Quantitative Test Kits were used for the measurement of aflatoxin, deoxynivalenol, fumonisin, T-2 and zearalenone.. The detection limits for each of them were 5 ppb, 0.5 ppm, 1 ppm, 0.025 ppm and 0.025 ppm. Data reported for WDG was on a dry weight basis.

Results

All of the mycotoxins except deoxynivalenol examined in both co-products, DDGS and WDS, were well below the FDA recommendations or guidelines for each mycotoxin in animal feed. The results of this study can be found in Tables 8a and 8b.

Aflatoxin: Average aflatoxin concentration was 4.609 ppb, with a maximum concentration of 7.097 ppb. All 30 DDGS samples tested for aflatoxin were below the FDA action levels for all animal species. The average (2.170 ppb) and maximum concentrations (6.785 ppb) of aflatoxin found in 28 WDG samples were also below the FDA action levels for all animal species.

Deoxynivalenol: The average concentration of deoxynivalenol in the 54 DDGS samples was 3.620 ppm, which was below the FDA advisory level for any animal diet, but the maximum concentration of 7.743 ppm in DDGS was higher than the advisory level for swine, cattle, chickens and other animals. At this level, the inclusion of the deoxynivalenol contaminated DDGS should not exceed 20% of the animal diet. It is unknown the proportion of DDGS samples that contained concentrations greater than 5 ppm. As for the 44 WDG samples analyzed for deoxynivalenol, the average concentration of was 1.905 ppm, and the maximum level was 4.257 ppm, with both levels lower than the FDA advisory level for any animal diet.

Fumonisin: Average fumonisin concentration was 0.740 ppm, with a maximum concentration of 1.959 ppm among the 20 DDGS samples tested for fumonisins, and were below the FDA recommendation levels for all animal species. The average (0.688 ppm) and maximum concentrations (1.729 ppm) of fumonisin found in 27 WDG samples were also below the FDA action levels for all animal species.

T-2 toxin: For DDGS samples, the average level of T-2 was 0.031 ppm and the maximum level was 0.065 ppm, while for WDG samples, the average level was 0.122 ppm and the maximum level was 0.240 ppm.

Zearalenone: For DDGS samples, the average level of zearalenone was 0.239 ppm and the maximum level was 0.510 ppm, while for WDG samples, the average level was 0.374 ppm and the maximum level was 0.869 ppm.

This study showed that aflatoxin, deoxynivalenol, fumonisin, T-2 toxin and zearalenone were detected in DDGS and WDG samples submitted to Dairy One Forage Lab from 2000 to 2007, however, the levels of aflatoxin, fumonisins, T-2 toxin and zearalenone were well below the maximum tolerable guidelines for use in animal feed, while a certain portion of the samples contained deoxynivalenol higher than 5 ppm but lower than 10 ppm. The results of this study are difficult to interpret because the geographic distribution of DDGS sources were not adequately described. In addition, the author did not indicate what aflatoxins or fumonisins they measured.

Table 8a. Results of Mycotoxin Concentrations in DDGS (Garcia et al, 2008)

Mycotoxins	Number of Samples Submitted	Average Level (of all samples)	Maximum Level	Percentage of Samples above the Lowest FDA Level
Aflatoxin (ppb)	30	4.609	7.097	0 %
Deoxynivalenol (ppm)	54	3.620	7.743	N.A.
Fumonisin (ppm)	20	0.740	1.959	0 %
T-2 toxin (ppm)	11	0.031	0.065	N.A.
Zearalenone (ppm)	16	0.239	0.510	N.A.

Table 8b. Results of Mycotoxin Concentrations in WDG (Garcia et al, 2008)

Mycotoxins	Number of Samples Submitted	Average Level (of all samples)	Maximum Level	Percentage of Samples above the Lowest FDA Level
Aflatoxin (ppb)	28	2.170	6.785	0 %
Deoxynivalenol (ppm)	44	1.905	4.257	0 %
Fumonisin (ppm)	27	0.688	1.729	0 %
T-2 toxin (ppm)	14	0.122	0.240	N.A.
Zearalenone (ppm)	14	0.374	0.869	N.A.

Summary

We have examined the available data relative to concentrations of various mycotoxins, both regulated and unregulated by the FDA, in DDGS from two independent studies, one research study and two publications. From these results all concentrations of mycotoxins in DDGS, and also in WGS from one study, were generally below the FDA regulations for the specific mycotoxins. Only in a couple of exceptions were the concentrations of deoxynivalenol or fumonisins either at, or slightly above, the recommendations for selected sensitive animal species, and in those instances the occurrence rate was lower than 10% of the samples tested. These concentrations could fall well below any harmful concentration when the DDGS are blended with other ingredients to make up the overall animal diet.

The methodology for analysis of mycotoxins in grain and co-products such as DDGS are quite sensitive and the fact that mycotoxins are detectable has no relationship with their toxicity in any animal species. The dosage makes the toxin, and the animals that are fed DDGS in today's marketing of this co-product are not as sensitive as perhaps other animal species such as pet species and humans.

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