DOI:10.1021/jf901186r

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

The Occurrence and Concentration of Mycotoxins in U.S. Distillers Dried Grains with Solubles

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To provide a scientific sound assessment of the prevalence and levels of mycotoxins in U.S. distillers' dried grains with solubles (DDGS), we measured mainly aflatoxins, deoxynivalenol, fumonisins, T-2 toxin, and zearalenone in 235 DDGS samples collected from 20 ethanol plants in the midwestern United States and 23 export shipping containers from 2006 to 2008 using state-of-the-art analytical methodologies. The results suggested that (1) none of the samples contained aflatoxins or deoxynivalenol levels higher than the U.S. Food and Drug Administration (FDA) guidelines for use in animal feed; (2) 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 fumonisins lower than FDA guidelines for use in animal feed; (3) none of the samples contained T-2 toxins higher than the detection limit, and no FDA guidance levels are available for T-2 toxins; (4) most samples contained zearalenone levels lower than the detection limit, and no FDA guidance levels are available for zearalenone; and (5) the containers used for export shipping of DDGS did not seem to contribute to mycotoxin production. This study was based on representative DDGS samples from the U.S. ethanol industry, and the data were collected using reference methods. This study provided a comprehensive and scientifically sound assessment of the occurrence and levels of mycotoxins in DDGS from the U.S. ethanol industry.

KEYWORDS: DDGS; mycotoxins; aflatoxins; deoxynivalenol; fumonisins; T-2 toxins; zearalenone

INTRODUCTION

Mycotoxins are unavoidable contaminants in crops; therefore, they occur in commodities entering the marketing chain including grains used in ethanol production (*1*). Currently, corn (maize) is the primary commodity used for the production of ethanol in the United States. However, depending on the 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 distillers' dried grains with solubles (DDGS). Several mycotoxins can potentially be found in corn including aflatoxins, deoxynivalenol (DON), fumonisins, T-2 toxins, and zearalenone (*1*). Most of these toxins can occur in corn, preharvest, and are 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 (*2*−*4*). 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 preharvest 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 (*5*).

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 (*6*). However, the remaining coproduct 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 (*7*, *8*). 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.

Recently, numerous reports have shown or cited data on detectable mycotoxins in DDGS, and concerns have been raised about using U.S. DDGS as animal feed (*9*−*11*). The report by Rodrigues (*10*) showed that 99% of the 103 DDGS samples that they studied contained at least one detectable mycotoxin, with 8% containing detectable aflatoxins, 64% containing detectable DON, 87% containing detectable fumonisins, 26% containing T-2 toxins, and 92% containing detectable zearalenone. Among

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the 103 DDGS samples, 67% were from the United States and 33% were from Asia. The report did not indicate how many of the samples with detectable levels of mycotoxins were from the United States. Furthermore, because all values for the mycotoxins were reported as maxima and averages, it was difficult to determine the percentage of these samples that contained concentrations of mycotoxins that were above the U.S. Food and Drug Administration (FDA) action levels or recommended maximum tolerable levels for use in animal feed (*12*). This report could mislead the international DDGS market in evaluating the quality of DDGS produced from the United States.

Another report by Garcia et al. (*9*) reviewed data generated by Dairy One Forage Laboratory in Ithaca, New York. The data were based on samples of DDGS and wet distillers' grains (WDG) submitted to the lab from 2000 to 2007 for mycotoxin analysis. No information was provided regarding the geographic distribution of the samples or how the DDGS samples were collected (*13*). All of the mycotoxins except DON examined in both coproducts, DDGS and WDG, were well below the FDA recommendations or guidelines for each mycotoxin in animal feed. The average concentration of DON 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 DON contaminated DDGS should not exceed 20% of the animal diet. Again, it was difficult to determine the percentage of the samples that contained concentrations of mycotoxins that were above the FDA advisory level.

In general, the reports cited above did not reveal the sampling source or sampling procedure for their studies; therefore, the representativeness of those studies was questionable and the conclusions from those studies were not scientifically sound. To provide a scientific sound assessment of the prevalence and levels of mycotoxins in U.S. DDGS, we measured various mycotoxins, including aflatoxins, DON, fumonisins, T-2 toxins, and zearalenone, in 235 DDGS samples collected from 20 ethanol plants in the midwestern United States and 23 export shipping containers from 2006 to 2008. Our goal was to share with the scientific community and the end users of DDGS the prevalence and levels of mycotoxins in U.S. DDGS based on a retractable sampling source and testing procedure. We ensured that the samples were representative of the ethanol industry in the United States and that the conclusion was prudent. Finally, we tried to evaluate the meaning of the results relative to the potential for toxicity in animals to which the DDGS might likely be fed.

MATERIALS AND METHODS

Experiment 1. This part of the study was led by the National Corn-to-Ethanol Research Center (NCERC).

Sample Collection. Twenty DDGS samples from the DDGS Library of NCERC (*14*) 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) between May of 2007 and May of 2008. 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 °C.

Sample Testing. The mycotoxin tests were performed at the Trilogy Analytical Laboratories. Samples were analyzed for aflatoxins B_1, B_2, G_1, and G_2, DON, fumonisins B_1, B_2, and B_3, and zearalenone by high-performance liquid chromatography (HPLC) and for T-2 toxin by thin-layer chromatography (TLC). Aflatoxins B_1, B_2, G_1, and G_2 were detected after extraction with acetonitrile/water (84/16), isolation using a solid-phase cleanup column (Trilogy TC-M160), and detection with a fluorescence detector with a Kobra cell for postcolumn derivatization (*15*). Fumonisins B_1, B_2, and B_3 were detected after extraction with methanol/water (3/1), isolation using an immunoaffinity cleanup column, and detection with a fluorescence detector with naphthalene dicarboxaldehyde (NDA) for precolumn derivatization (*16*). DON was detected after extraction with acetonitrile/water (84/16), isolation using a combination of solid-phase (Trilogy TC-M160 and TC-C210) and immunoaffinity cleanup columns, and detection with a UV detector (*17*). T-2 toxin was detected after extraction with acetonitrile/water (84/16), isolation using a combination of solid-phase cleanup columns (Trilogy TC-M160 and TC-C210), and TLC detection (*18*). Zearelenone was detected after extraction with acetonitrile/water (84/16), isolation using a combination of solid-phase (Trilogy TC-M160) and immunoaffinity cleanup columns, and detection with a fluorescence detector (*19*). The detection limits for the tests were 1 ppb for each aflatoxin, 0.1 ppm for DON, 0.1 ppm for each fumonisin, 0.1 ppm for T-2 toxin, and 0.05 ppm for zearalenone.

Experiment 2. This part of the study was led by an ethanol producer in the midwest of the United States.

Sample Collection. From February of 2006 to November of 2007, DDGS samples were collected from two ethanol plants (plants A and B) owned by the ethanol producer for mycotoxin tests. More than one DDGS sample was collected on a monthly basis from each ethanol plant and sent to the Midwest Laboratories for mycotoxin testing. Between February of 2008 and July of 2008, combined DDGS samples from four ethanol plants owned by the ethanol producer were collected weekly and sent to the MVTL Laboratories for mycotoxin testing.

Sample Testing. Samples were analyzed for aflatoxins B_1, B_2, G_1, and G_2, DON, fumonisins B_1, B_2, and B_3, T-2 toxin, and zearalenone. The methodology utilized by the Midwest Laboratories was liquid chromatography/mass spectrometry (LC/MS). On the basis of numerous methods from the literature, a proprietary method was developed and run as follows: the analyses of aflatoxins B_1, B_2, G_1, and G_2, fumonisins B_1 and B_2, T-2 toxin, and zearalenone were carried out using a methanol/water extraction solution to dissolve the potential mycotoxins in samples. After they were vortexed and centrifuged, the extracts were passed through an affinity column. The affinity column was washed with a phosphate buffer and eluted with methanol. The extracts were analyzed by LC/MS. For DON, except using deionized water for extraction, the rest of the procedure was the same as for the other mycotoxins. Detection limits for the tests were 1 ppb for each aflatoxin, 0.1 ppm for DON, each fumonisin, and T-2 toxin, and 0.05 ppm for zearalenone.

The methodology utilized by the MVTL Laboratories was HPLC. For aflatoxins B_1, B_2, G_1, and G_2 and zearalenone, the sample was extracted with 70/30 methanol/water, and the extract was run through a Vicam AOZ immunoaffinity column and detected by a fluorescence detector. 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 zearalenone (*20, 21*). For fumonisins B_1 and B_2 measurement, the sample was extracted with 25/25/50 methanol/acetonitrile/water; after precolumn derivitization with *o*-phthalaldehyde, the extract was run through a Vicam fumonitest immunoaffinity column and detected by a fluorescence detector. Detection limits for the tests were 0.2 ppm for fumonisins B_1 and B_2 (*16*). For DON, the sample was extracted with deionized water, and the extract was run through a Vicam DONtest immunoaffinity column and detected by a UV/vis detector. The detection limit was 0.2 ppm for DON.

Experiment 3. This part of the study was led by Iowa State University, Veterinary Diagnostic Lab, and Novecta LLC.

Sample Collection. 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 during the Taiwanese winter season of 2006. The study included seven DDGS samples coming directly from different U.S. 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 (winter season). The samples were then shipped back to the Veterinary Diagnostic Lab at Iowa State

University for analysis. Phase II was conducted during the Taiwanese 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 of the shipping containers was to be done using the Kansas State University probe technique (*22*). However, because of safety concerns, all 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 subsampled into 400−500 g samples before shipment. All sampling in the United States was overseen by U.S. Department of Agriculture 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 Taiwanese origin so that comparison of data could be drawn.

Sample Testing. All analyses were performed at the Veterinary Diagnostic Lab at Iowa State University (Ames, IA). 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 aflatoxins (B_1, B_2, G_1, and G_2), DON, total fumonisin, T-2 toxin, and zearalenone/zearalenol by TLC. The detection limits of the TLC method were 5 ppb for each aflatoxin, 0.5 ppm for DON, each fumonisin, and zearalenone, and 1 ppm for T-2 toxin. 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 aflatoxins, 1 ppm for DON, fumonisins, and zearalenone/zearalenol, and 2 ppm for T-2 toxin. For the samples with mycotoxin levels above the detection limit, a confirmatory test was performed using HPLC or GC (*23*). The detection limits for the HPLC method were 0.5 ppb for each aflatoxin and 0.1 ppm for total fumonisins and zearalenone, and the detection limits for GC method were 0.1 ppm for DON and 0.3 ppm for T-2 toxin.

RESULTS

The results for experiment 1 are listed in **Table 1**, the results for experiment 2 are listed in **Tables 2−4**, and those for experiment 3 are shown in **Tables 5−7**. In the following, we will present the results based on the individual mycotoxin tested.

Aflatoxins. The major fungus to produce aflatoxins in corn, is *Aspergillus flavus*. Corn becomes susceptible to aflatoxin formation during growth under drought conditions or in high moisture/humid storage (*24*).

For samples from experiment 1, aflatoxin B1 was detected in six DDGS samples with the highest level of 3.7 ppb. None of the other aflatoxin compounds, B_2, G_1, and G_2, was detected in any of the 20 samples (**Table 1**).

For samples from experiment 2, from plant A, aflatoxin B_1 was detected only in three DDGS samples. None of the other aflatoxin compounds, B_2, G_1, and G_2, was detected in any of the 69 samples (**Table 2**). For samples from plant B, aflatoxin B_1 was detected only in two DDGS samples. None of the other aflatoxin compounds, B_2, G_1, and G_2, was detected in any of the 16 samples (**Table 3**). For the combined samples from the four plants, aflatoxin B_2 was detected only in one DDGS (1.1 ppb). None of the aflatoxin compounds, B_1, B_2, G_1, and G_2, was detected in any of the other 76 samples (**Table 4**). The highest level of aflatoxin detected was 2.56 ppb.

None of the samples from experiment 3 was found to contain aflatoxins that exceeded the limit of detection of 5 ppb (**Tables 5−7**). Overall, none of the aflatoxins was detected in most DDGS samples, and the highest level was found to be 3.7 ppb.

DON. *Fusarium graminearum* is the principal DON-producing fungus in grains in the United States (*5*). DON may coexist with other toxins, like zearalenone. The organism survives on old infested residue left on the field from the previous season, where a cold moist condition is favorable for the fungus to grow on corn. Generally, storage is not considered a potential source for contamination if the corn is mature and is stored at a moisture level lower than 14% (*24*).

Table 1. Mycotoxins in DDGS[a]

	aflatoxin B_1 (ppb)	DON (ppm)	total fumonisins (ppm)	T-2 toxin (ppm)	zearalenone (ppb)
plant 1	ND	0.4	0.9	ND	74
plant 2	3.7	0.1	3.5	ND	98
plant 3[b]	ND	0.1	2.2	ND	ND
plant 3[b]	1.9	0.2	6.8	ND	72
plant 4[b]	3	0.2	8.6	ND	87
plant 4[b]	ND	0.2	4.5	ND	57
plant 5	ND	0.1	0.4	ND	ND
plant 6	ND	ND	0.7	ND	ND
plant 7[b]	ND	0.7	0.6	ND	ND
plant 7[b]	ND	0.4	0.7	ND	ND
plant 8	ND	1.1	0.6	ND	127
plant 9[b]	ND	0.9	0.7	ND	56
plant 9[b]	ND	0.8	1.1	ND	ND
plant 10	ND	0.1	2.9	ND	ND
plant 11[b]	ND	1.2	2.0	ND	143
plant 11[b]	ND	0.4	0.5	ND	53
plant 12[b]	1.6	ND	0.1	ND	ND
plant 12[b]	1.9	ND	ND	ND	ND
plant 13	1.1	ND	0.7	ND	ND
plant 14	ND	ND	0.1	ND	ND

[a] Results for experiment 1. The study was led by the NCERC, and samples were collected between 5/2007 and 5/2008. [b] Samples were sent to NCERC twice within a year.

For samples from experiment 1, DON was detected in 15 DDGS samples. The detected level of DON ranged from 0.1 to 1.2 ppm. For the six ethanol plants that submitted samples twice, the levels of DON were close to each other for each ethanol plant except one (plant 11, **Table 1**).

For samples from experiment 2, from plant A, DON was detected in 66 DDGS samples. The detected level of DON in DDGS ranged from 0.1 to 1.42 ppm, and no obvious temporal trend of DON content in DDGS was observed from 2/2006 to 11/2007 (**Table 2** and **Figure 1a**). For samples from plant B, DON was detected in all of the DDGS samples. The detected level of DON in DDGS ranged from 0.1 to 1.68 ppm, and no obvious temporal trend of DON content in DDGS was observed from 7/2006 to 11/2007 (**Table 3** and **Figure 1b**). For the combined samples from four plants, DON was detected in 77 DDGS samples. The level of DON stayed relatively stable around 0.5 ppb with very few spikes during the short sampling period of 7 months (2/2008−7/2008) (**Table 4** and **Figure 1c**).

Data from experiment 3 showed that four samples from phase I (two from the ethanol plants and two from the Taiwanese port) contained detectable DON levels. The maximum level detected was 3.4 ppm (**Table 5**). Overall, the majority of the DDGS samples tested contained DON higher than 0.1 ppm, and the highest level was found to be 1.68 ppm.

Table 2. Mycotoxins in DDGS[a]

sample no.	aflatoxin B_1 (ppb)	DON (ppm)	total fumonisins (ppm)	T-2 toxin (ppm)	zearalenone (ppb)
1	<1[b]	0.24	0.12	<0.1	<50
2	<1	0.21	0.13	<0.1	<50
3	<1	0.24	1.76	<0.1	57
4	<1	0.19	1.54	<0.1	93
5	<1	0.34	1.28	<0.1	78
6	<1	0.15	1.14	<0.1	<50
7	<1	0.12	1.3	<0.1	105
8	<1	1.05	1.17	<0.1	117
9	<1	1.24	1.12	<0.1	109
10	<1	0.4	1.95	<0.1	<50
11	<1	0.36	1.04	<0.1	<50
12	<1	0.3	1.64	<0.1	<50
13	<1	<0.1	1.71	<0.1	<50
14	<1	<0.1	1.45	<0.1	<50
15	<1	<0.1	0.62	<0.1	<50
16	<1	0.75	0.93	<0.1	<50
17	<1	0.72	2.36	<0.1	<50
18	<1	0.68	2.39	<0.1	<50
19	<1	0.54	0.56	<0.1	<50
20	<1	0.65	2.96	<0.1	<50
21	<1	0.67	3.5	<0.1	<50
22	<1	0.63	2.84	<0.1	<50
23	<1	0.64	2.28	<0.1	<50
24	<1	1.26	1.79	<0.1	<50
25	<1	1	1.42	<0.1	59
26	<1	0.42	0.53	<0.1	<50
27	<1	0.68	1.15	<0.1	<50
28	<1	0.75	2.16	<0.1	<50
29	<1	0.74	1	<0.1	<50
30	<1	0.86	1.1	<0.1	<50
31	<1	1.04	0.88	<0.1	50
32	<1	1.09	3.31	<0.1	59
33	1.89	1.42	3.59	<0.1	54
34	<1	0.8	0.87	<0.1	<50
35	<1	0.6	1.67	<0.1	74
36	<1	0.33	1.77	<0.1	<50
37	<1	0.56	1.24	<0.1	<50
38	<1	1.13	1.36	<0.1	<50
39	<1	0.65	0.66	<0.1	<50
40	2.56	0.19	4.69	<0.1	<50
41	<1	0.26	5.85	<0.1	<50
42	1.04	0.17	4.05	<0.1	91
43	<1	0.49	3.47	<0.1	64
44	<1	0.52	4.46	<0.1	75
45	<1	1.25	4.32	<0.1	123
46	<1	0.91	2.04	<0.1	<50
47	<1	0.74	1.84	<0.1	<50
48	<1	0.83	2.28	<0.1	<50
49	<1	0.66	2.62	<0.1	<50
50	<1	1	2.01	<0.1	<50
51	<1	1.36	5.88	<0.1	104
52	<1	1.33	4.19	<0.1	78
53	<1	0.6	3.01	<0.1	<50
54	<1	0.68	2.44	<0.1	74
55	<1	0.39	2.22	<0.1	85
56	<1	0.81	2.66	<0.1	58
57	<1	0.48	1.85	<0.1	52
58	<1	0.71	1.82	<0.1	<50
59	<1	0.84	1.83	<0.1	58
60	<1	0.88	2.82	<0.1	<50
61	<1	0.89	3.17	<0.1	<50
62	<1	0.99	2.46	<0.1	<50
63	<1	0.97	3.8	<0.1	<50
64	<1	0.72	3.36	<0.1	<50
65	<1	0.4	3.26	<0.1	<50
66	<1	0.38	3.74	<0.1	<50
67	<1	0.25	4.9	<0.1	<50
68	<1	0.48	4.77	<0.1	<50
69	<1	0.52	4.48	<0.1	<50

[a] Results for experiment 2. The study was led by an ethanol producer, and samples were collected from plant A about monthly between 2/2006 and 11/2007. [b] The symbol "<" indicates that the mycotoxin content in the sample is lower than the detection limit of the testing method.

Table 3. Mycotoxins in DDGS[a]

sample no.	aflatoxin B_1 (ppb)	DON (ppm)	total fumonisins (ppm)	T-2 toxin (ppm)	zearalenone (ppb)
1	<1	0.13	1.71	<0.1	<50
2	1.21	1.5	0.88	<0.1	59
3	<1	1.33	0.28	<0.1	<50
4	<1	1.1	1.65	<0.1	70
5	1.15	1.14	1.68	<0.1	111
6	<1	1.46	1.44	<0.1	113
7	<1	1.17	1.89	<0.1	84
8	<1	1.28	2.14	<0.1	60
9	<1	0.62	1.01	<0.1	<50
10	<1	1.09	1.15	<0.1	<50
11	<1	0.33	1.49	<0.1	<50
12	<1	0.47	2.77	<0.1	52
13	<1	0.69	1.36	<0.1	57
14	<1	1.17	2.31	<0.1	72
15	<1	1.68	1.17	<0.1	<50
16	<1	1.13	0.61	<0.1	<50

[a] Results for experiment 2. The study was led by an ethanol producer, and samples were collected from plant B about monthly between 7/2006 and 11/2007.

Table 4. Mycotoxins in DDGS[a]

sample no.	aflatoxin B_1 (ppb)	DON (ppm)	total fumonisins (ppm)	zearalenone (ppb)
1	<3	0.2	2.3	<0.2
2	<3	0.4	2.5	<0.2
3	<3	0.3	4.3	<0.2
4	<3	0.3	2.6	<0.2
5	<3	0.5	1.4	<0.2
6	<3	0.3	1.6	<0.2
7	<3	0.5	1.2	<0.2
8	<3	0.4	4.6	<0.2
9	<3	0.3	2.3	<0.2
10	<3	0.3	1.8	<0.2
11	<3	0.6	1.3	<0.2
12	<3	0.3	3.3	<0.2
13	<3	0.4	2.2	<0.2
14	<3	0.4	1.5	<0.2
15	<3	0.3	5.5	<0.2
16	<3	0.4	2.9	<0.2
17	<3	0.6	1.3	<0.2
18	<3	1.3	1.5	<0.2
19	<3	0.6	2.8	<0.2
20	<3	1.9	1.6	<0.2
21	<3	0.7	6.5	<0.2
22	<3	0.5	1.9	<0.2
23	<3	0.4	5.9	<0.2
24	<3	0.2	2.9	<0.2
25	<3	0.4	1.9	<0.2
26	<3	0.5	2.4	<0.2
27	<3	0.4	6.3	<0.2
28	<3	0.7	2.1	<0.2
29	<3	0.4	3.3	<0.2
30	<3	0.4	4.2	<0.2
31	<3	0.4	6.4	<0.2
32	<3	0.6	1.3	<0.2
33	<3	0.4	1.5	<0.2
34	<3	0.4	5	<0.2
35	<3	0.6	1.3	<0.2
36	<3	0.5	1.1	<0.2
37	<3	0.4	7.2	<0.2
38	<3	0.7	1.1	<0.2
39	<3	0.4	<0.2	<0.2
40	<3	0.6	1.5	<0.2
41	<3	0.4	1.7	<0.2
42	<3	0.4	5.5	<0.2
43	<3	0.6	0.9	<0.2
44	<3	0.5	1.1	<0.2
45	<3	0.4	4	<0.2
46	<3	0.4	2.2	<0.2
47	<3	0.7	1.1	<0.2
48	<3	0.4	2.6	<0.2
49	<3	0.4	4.9	<0.2
50	<3	0.7	1.1	<0.2
51	<3	0.5	1.5	<0.2
52	<3	0.5	2.5	<0.2
53	<3	0.3	4.8	<0.2
54	<3	0.4	2.4	<0.2
55	<3	0.3	4.8	<0.2
56	<3	0.4	2.7	<0.2
57	<3	0.3	4.6	<0.2
58	<3	0.5	1.8	<0.2
59	<3	0.4	4.3	<0.2
60	<3	0.3	2.5	<0.2
61	<3	0.5	1.6	<0.2
62	<3	0.5	2.3	<0.2
63	<3	0.4	3.1	<0.2
64	<3	0.5	1.5	<0.2
65	<3	0.3	3.3	<0.2
66	<3	0.5	2.3	<0.2
67	<3	0.3	5.4	<0.2
68	<3	0.3	1.9	<0.2
69	<3	0.6	1	<0.2
70	<3	0.3	1.2	<0.2
71	<3	0.3	4.3	<0.2
72	<3	0.6	0.8	<0.2
73	<3	0.4	1.5	<0.2
74	<3	0.6	1.5	<0.2
75	<3	0.4	1.8	<0.2
76	<3	0.4	4.1	<0.2
77	<3	0.5	<0.2	<0.2

[a] Results for experiment 2. The study was led by an ethanol producer, and combined samples were collected from four ethanol plants weekly between 2/2008 and 7/2008.

Fumonisins. The major producer, *F. verticillioides*, is capable of producing the fumonisins, mainly FB_1, FB_2, and FB_3 (*25*). Corn is the major commodity affected by the fungi that produce the toxins. The exact conditions for causing this are unknown, but it is suggested that drought stress followed by warm, wet weather during flowering seems to be important. It is reported that the organism is present virtually in every seed and is present in the corn plant throughout its growth, and sometimes, there is a considerable amount of fumonisins present in symptomless kernels of corn. Because the discovery of this toxin was fairly recent (1988), there is considerable information lacking for this toxin (*24*).

For samples from experiment 1, fumonisins were detected in 19 DDGS samples. The detected level of fumonisins ranged from 0.1 to 8.6 ppm. For the six ethanol plants that submitted samples twice, the level of fumonisins varied a great deal from each other—as high as three times (**Table 1**).

For samples from plant A, fumonisins were detected in all of the 69 DDGS samples. The second half of the samples seemed to have a slightly higher level of fumonisins than the first half (**Figure 1d**). The level of total fumonisins in DDGS fluctuated between 0.1 and 5.88 ppm (**Table 2**). For samples from plant B, fumonisins were detected in all of the 16 DDGS samples. No obviously temporal trend of fumonisins content in DDGS was

Table 5. Mycotoxins in DDGS[a]

mycotoxins	no. of samples submitted	minimum level	maximum level	average level (of all samples)	percentage of samples above the lowest FDA level
aflatoxins (ppb)	7	<5	<5	0	0
DON (ppm)	7	<0.1	3.4	0.6	0
fumonisins (ppm)	7	1.8	2.9	2.3	0
T-2 toxin (ppm)	7	<1	<1	0	NA
zearalenone/ zearalenol (ppm)	7	<0.5	<0.5	0	NA

[a] Results for experiment 3, phase I. The study was led by ISU, and samples were collected directly from ethanol plants in the Taiwanese winter season of 2006.

Table 6. Mycotoxins in DDGS[a]

mycotoxins	no. of samples submitted	minimum level	maximum level	average level (of all samples)	percentage of samples above the lowest FDA level
aflatoxins (ppb)	11[b]	<5	<5	0	0
	11[c]	<5	<5	0	0
DON (ppm)	11	<0.5	<0.5	0	0
	11	<0.1	1.0	0.1	0
fumonisins (ppm)	11	0.7	2.4	1.9	0
	11	0.7	2.0	1.2	0
T-2 toxin (ppm)	11	<1	<1	0	NA
	11	<1	<1	0	NA
zearalenone/ zearalenol (ppm)	11	<0.5	<0.5	0	NA
	11	<0.5	<0.5	0	NA

[a] Results for experiment 3, phase I. The study was led by ISU, and the samples were collected from U.S. port containers and from Taiwanese port containers in the Taiwanese winter season of 2006. [b] Data for samples from U.S. port containers. [c] Data for samples from Taiwanese port containers.

Table 7. Mycotoxins in DDGS[a]

mycotoxins	number of samples submitted	minimum level	maximum level	average level (of all samples)	percentage of samples above the lowest FDA level
aflatoxins (ppb)	12[b]	<5	<5	0	0
	12[c]	<5	<5	0	0
DON (ppm)	12	<0.5	<0.5	0	0
	12	<0.5	<0.5	0	0
fumonisins (ppm)	12	0.5	1.4	0.9	0
	12	0.4	2.4	1.5	0
T-2 toxin (ppm)	12	<1	<1	0	NA
	12	<1	<1	0	NA
zearalenone/ zearalenol (ppm)	12	<0.5	<0.5	0	NA
	12	<0.5	<0.5	0	NA

[a] Results for experiment 3, phase II. The study was led by ISU, and samples were collected from U.S. port containers and from Taiwanese port containers in the Taiwanese summer season of 2007. [b] Data for samples from U.S. port containers. [c] Data for samples from Taiwanese port containers.

observed from 2/2006 to 11/2007 (**Figure 1e**). The level of total fumonisins in DDGS fluctuated between 0.61 and 2.77 ppm (**Table 3**). For the combined samples from the four plants, fumonisins were detected in 75 DDGS samples. No obviously temporal trend of fumonisins content in DDGS has been seen from 2/2008 to 7/2008 (**Figure 1f**). The level of total fumonisins in DDGS fluctuates between 0.1 to 7.2 ppm (**Table 4**).

Data from experiment 3 showed that all 53 samples contained detectable fumonisins; the maximum level of fumonisins detected in a sample from phase I was 2.9 ppm (**Table 5**) and from phase II was 2.4 ppm (**Table 7**). In the phase I study, the average level of fumonisins was 2.3 ppm for the samples from the ethanol plants (**Table 5**), 1.9 ppm for the samples from the U.S. port (**Table 6**), and 1.2 ppm for the samples from the Taiwanese port (**Table 6**). In the phase II study, the average level of fumonisins was 0.9 ppm for the samples from the U.S. port (**Table 7**) and 1.5 ppm for the samples from the Taiwanese port (**Table 7**). Statistical analysis of the data showed no increase in fumonisins observed in the shipment of DDGS from the United States to Taiwan.

Overall, almost all of the DDGS samples tested contained detectable level of fumonisins. The highest level was found to be 8.6 ppm.

T-2 Toxin. This mycotoxin is a member of fungal metabolites known as the trichothecenes. *Fusarium sporotrichioides* is the principal fungus responsible for the production of T-2 toxin. The production of T-2 toxin is the greatest with increased humidity and temperatures of 6−24 °C (*5*). None of the DDGS samples tested in this study were found to contain levels above the detection limit of 0.1 ppm.

Zearalenone. This is an estrogenic fungal metabolite. The major fungus responsible for producing this toxin is *Fusarium graminearum* (*5*). A moist and cool growing condition is favorable for this fungus to grow for DON. For storage, controlling moisture lower than 14% is important to avoid contamination.

For the samples from experiment 1, about 50% of the DDGS was found to contain zearalenone higher than 50 ppb, and the detected level ranged form 53 to 143 ppb (**Table 1**). For the samples from plant A, less than 40% of the DDGS was found to contain zearalenone higher than 50 ppb, which is the detection limit. The detected level of zearalenone in DDGS ranged form 52 to 123 ppb (**Table 2**). For the samples from plant B, similar to the situation with plant A, about 50% of the DDGS was found to contain zearalenone higher than 50 ppb. The detected level of zearalenone ranged form 52 to 113 ppb (**Table 3**). All of the

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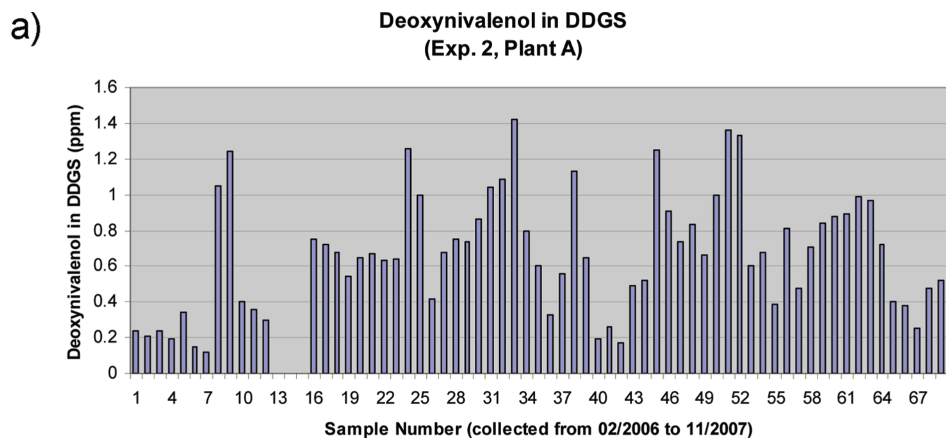

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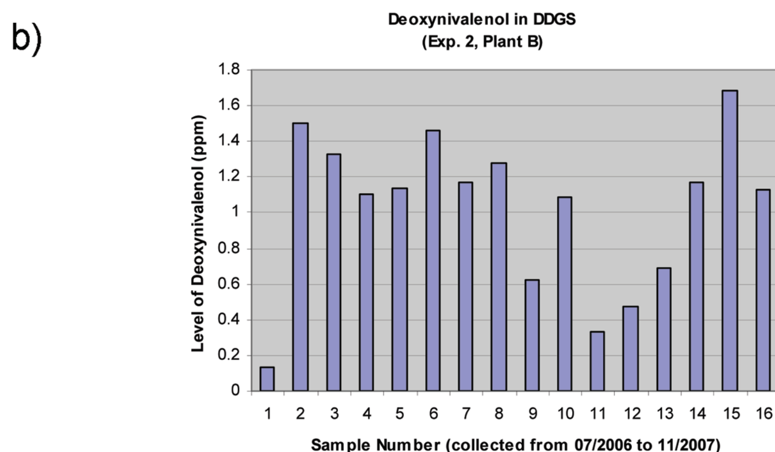

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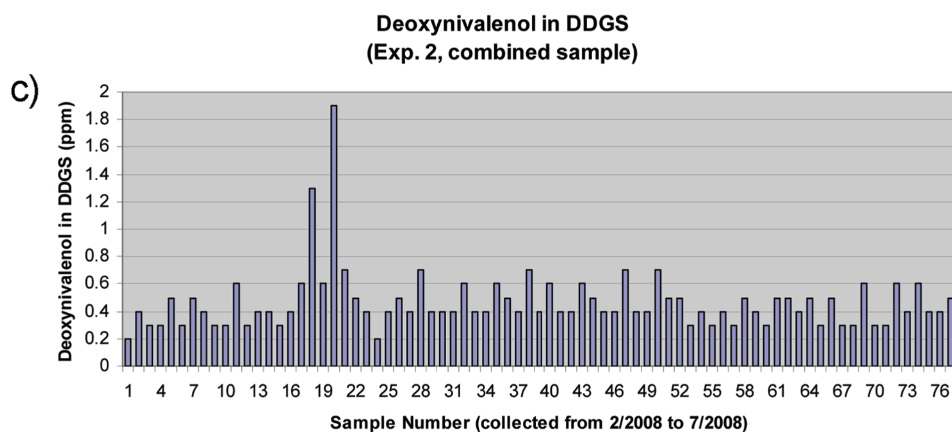

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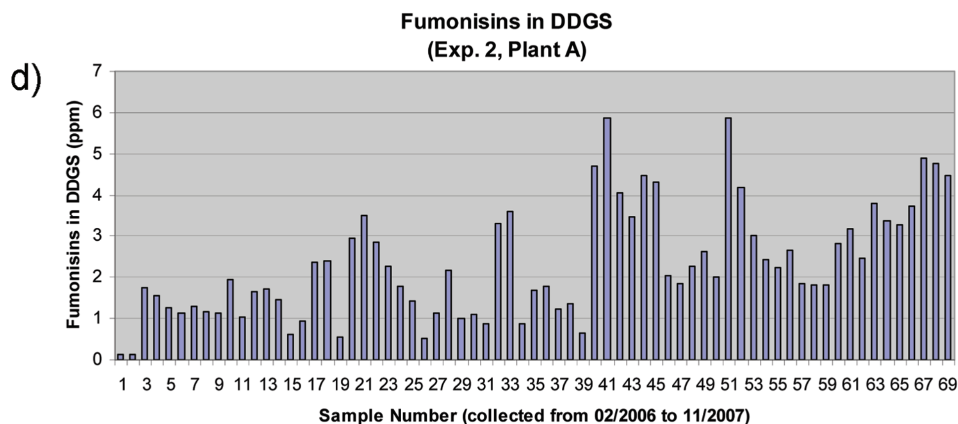

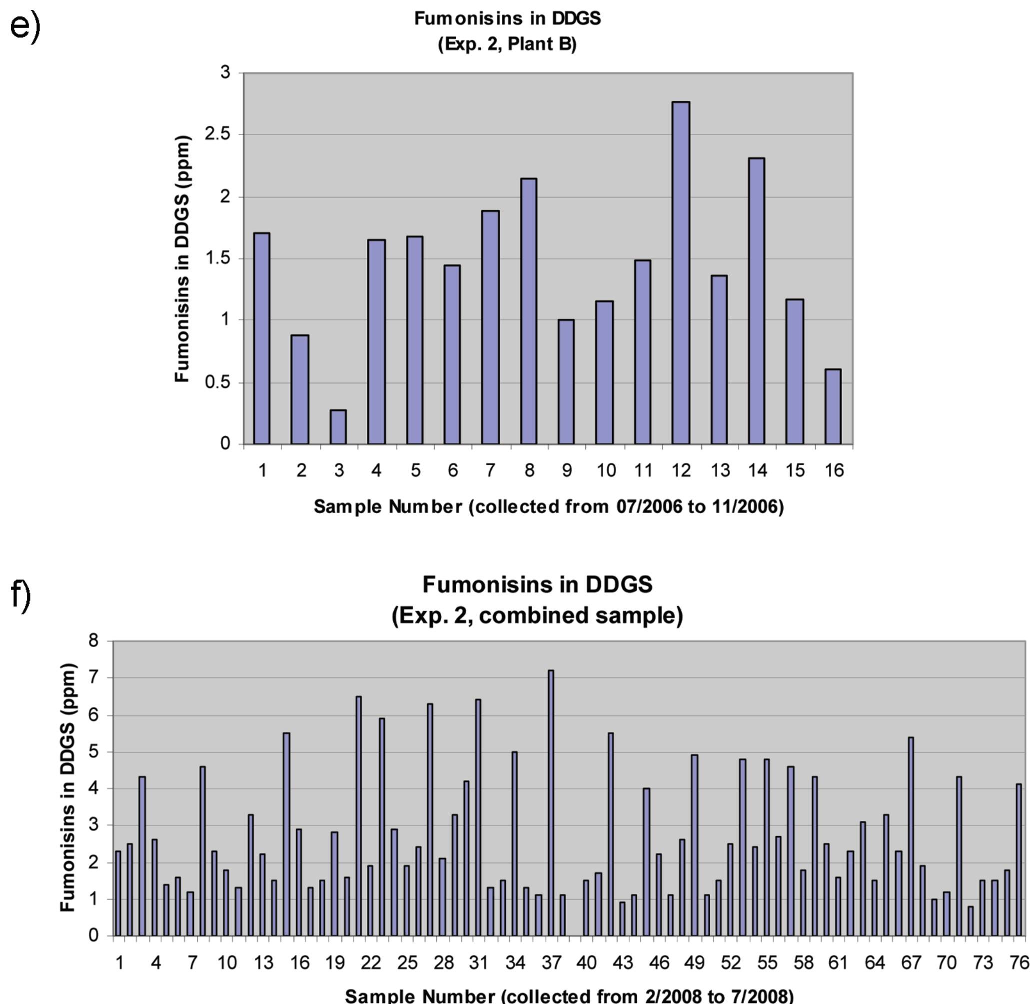

Figure 1. (**a**) Temporal change of DON in DDGS. Samples were from plant A of experiment 2. (**b**) Temporal change of DON in DDGS. Samples were from plant B of experiment 2. (**c**) Temporal change of DON in DDGS. Samples were combined from four plants from experiment 2. (**d**) Temporal change of fumonisins in DDGS. Samples were from plant A of experiment 2. (**e**) Temporal change of fumonisins in DDGS. Samples were from plant B of experiment 2. (**f**) Temporal change of fumonisins in DDGS. Samples were combined from four plants from experiment 2.

combined samples from the four plants that were tested resulted in a zearalenone level lower than 200 ppb (**Table 4**).

None of the 53 samples tested for the DDGS shipment had a level of zearalenone that exceeded the limit of detection of 0.5 ppm. Overall, almost all of the DDGS samples tested contained a detectable level of zearalenone. The highest level was found to be 143 ppb.

DISCUSSION

Mycotoxin Detection vs Toxicity. The presence of mycotoxins in feed grains or ingredients may cause illness and death in livestock. They also pose a potential hazard to human health because toxicologically, they can be carcinogenic and immunosuppressive. The most common symptoms in swine that are fed contaminated feed include depressed growth, infertility, decreased litter size, low piglet birth weights, immunosuppression, liver damage, oral lesions and tremors, etc. Chronic intoxication can result when low-level, long-term exposure to a single and/or multiple mycotoxins occurs (*5*). Because of the potential hazard, the FDA has established regulatory levels of mycotoxins for the use of feed ingredients as animal feed. Action levels for aflatoxins in animal feed were established for different animals and at different production stages in August, 2000. The FDA action level represents the minimum limit at which the FDA can take legal action to remove feed ingredients from the market. **Table 8** shows the action levels established for aflatoxins in animal feed, the advisory levels for DON in animal feeds, and the recommended maximum levels for fumonisins in animal feeds set by the FDA in 2001 (*12*). No action levels, advisory levels, or guidance levels for T-2 toxin or zearalenone are available from the FDA.

When comparing the results from **Tables 1−7** with FDA guidelines, we found that none of the 235 DDGS samples contained aflatoxins or DON levels higher than FDA guidelines for use in animal feed, and no more than 10% of the 235 samples contained fumonisins levels higher than the recommendation for feeding equids and rabbits; the rest of the samples contained fumonisins lower than FDA guidelines for use in animal feed. For those 10% DDGS above the FDA guideline level for fumonisins, the concentrations could fall well below any harmful concentration when the DDGS are blended with other ingredients to make up the overall animal diet. Although no FDA guidance levels are available for T-2 toxin, none of the samples contained T-2 toxin higher than the detection limit, and more than 50% of the DDGS samples contained zearalenone levels lower than detection limit.

The methodology for the analysis of mycotoxins in grain and coproducts such as DDGS has improved greatly in the past 10 years (*26*). The test kits for rapid and on-site determination of mycotoxins are more sensitive, and the confirmatory method of HPLC has improved greatly, including sample extraction and cleanup technology for higher sensitivity. Finally, the introduction

Table 8. FDA Action Levels for Mycotoxins in Feed Ingredients

animals	action levels (ppb)
aflatoxins	
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
DON	
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
fumonisins	
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

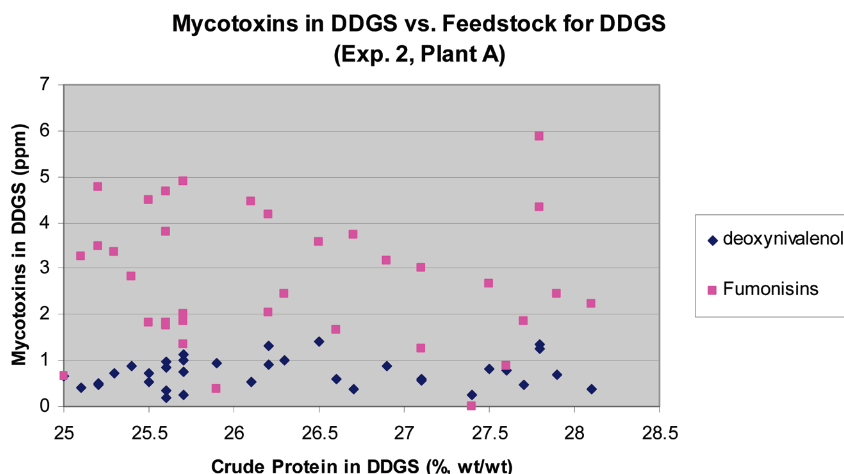

Figure 2. Mycotoxins (DON and fumonisins) in DDGS vs crude protein in DDGS, in search for any geographic dependence of the mycotoxins in DDGS (data were from experiment 2, but they are representative of the data from the other two experiments).

of LC/MS has gained considerable attention for its ability to simultaneously detect and identify multimycotoxins in animal feed. This method provides unambiguous confirmation of the molecular identity, uses simple extraction with little or no cleanup, and has high selectivity and sensitivity. The detection limits for mycotoxins in DDGS have become lower, and the fact that mycotoxins are detectable has no relationship with their toxicity in any animal species.

Temporal and Geographical Distribution of Mycotoxins in DDGS. It is well-known that mycotoxins in DDGS mainly come from contaminated corn, which is the currently major feedstock for ethanol production. DDGS storage could be another factor contributing to the increase of mycotoxins in DDGS. Because the DDGS samples tested for this study were collected immediately after they were produced, it is reasonable to assume that the mycotoxins in DDGS collected for this study were mainly from the corn used in the process.

DON and Zearalenone. The levels of DON and zearalenone in DDGS fluctuated randomly for plants A and B in about 2 years of a sampling period. When plotting the level of DON against crude protein content in DDGS samples from experiments 1, 2, and 3, there is no obvious strong correlation (**Figure 2**, showing an example from experiment 2). Crude protein is considered to be more related to feedstock, which could be geographically related (*14*). Therefore, we did not observe a strong correlation between DON in DDGS and time or DON in DDGS and geographic location.

When plotting the level of DON against zearalenone for samples from experiments 1 and 2, there was no obvious strong correlation (**Figure 3**, showing an example from experiment 2); therefore, we did not observe any trend to show that the higher the level of DON in DDGS, the higher the level of zearalenone in the same DDGS samples.

In Summary. We have examined the levels of various mycotoxins, both regulated and unregulated by the FDA, in DDGS from 20 ethanol plants during three crop years and in DDGS in oversea shipment containers. From these results, all concentrations of mycotoxins in DDGS were generally below the FDA regulations for the specific mycotoxins. Only in a couple of exceptions were the concentrations of DON 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

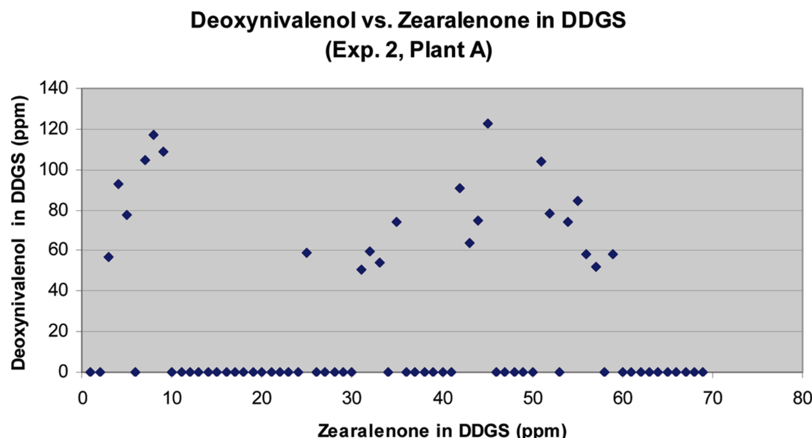

Figure 3. DON in DDGS vs zearalenone in DDGS (data were from experiment 2, but they are representative of the data from the other two experiments).

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. In this study, containers used for export shipping of DDGS do not seem to contribute to mycotoxin production (concentration).

The methodology for analysis of mycotoxins in grain and coproducts such as DDGS is 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 coproduct are not as sensitive as perhaps other animal species such as pet species and humans.

ACKNOWLEDGMENT

We thank the following individuals for help with this paper: Gary Delong, Barb Randle, and Ines Rodrigues. We thank the U.S. Grains Council for providing the financial support for this study. Most of the material presented in this paper was published in the Asian Feed Magazine—March/April, 2009, without recognition of the authors, and we have obtained permission from the Asian Feed Magazine to reproduce the work here.

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Received April 8, 2009. Revised manuscript received August 27, 2009. Accepted September 3, 2009.