World Mycotoxin Journal, February 2012; 5 (1): 83-88

Wageningen Academic Publishers

A comprehensive survey on the occurrence of mycotoxins in maize dried distillers' grain and solubles sourced worldwide

I. Rodrigues[1] and L.J. Chin[2]

[1]BIOMIN Holding GmbH, Industriestrasse 21, 3130 Herzogenburg, Austria; [2]ROMER Labs Singapore Pte Ltd, 3791 Jalan Bukit Merah, #08-08 e-Centre@Redhill, Singapore 159471, Singapore; office@biomin.net

Received: 14 April 2011 / Accepted: 10 August 2011

Abstract

As cereal and protein sources suffer a price increase worldwide, the use of alternative ingredients in feeds has become increasingly appealing to the animal industry. Dried distillers' grain and solubles (DDGS) have been one of the ingredients which demand has dramatically increased over the last few years. In fact, the supply of maize DDGS is expected to increase to about 38.6 mmt by 2019-2020. The presence of mycotoxins in these by-products has been a matter of concern raised by their ubiquitous use. A rule of thumb typically used in the field is that the concentration of mycotoxins in the final by-product is about three times higher than that of the original raw material, which may be scientifically backed up by the fact that the remaining portions within the by-product are those which had a higher concentration of mycotoxins (outer portions of the grain). This paper is the outcome of a five-year study during which a total of 409 maize DDGS samples sourced worldwide were analysed for the mycotoxins of major importance which cause the great negative impacts in animal performance and health: aflatoxins (a sum of aflatoxin B_1, B_2, G_1, G_2), zearalenone, deoxynivalenol, a sum fumonisins B_1 and B_2 and ochratoxin A. From the total of samples tested, only 2% of the analysed DDGS showed contamination levels below the limit of detection (negative samples). 6% of samples had the presence of 1 mycotoxin only and the majority (92% of the samples) was contaminated with 2 or more mycotoxins.

Keywords: DDGS, aflatoxins, zearalenone, deoxynivalenol, fumonisins, ochratoxin A

1. Introduction

By December 2006, Kyoto's Protocol had already been signed by 169 countries worldwide. As described on Article 2 of the United Nations Framework Convention on Climate Change, the final objective of such agreement is the stabilisation of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system (UNFCCC, 1994). According to data collected in the United States, one of the top 5 green house emitters, transportation activities account for the second largest portion of gas emissions (US EPA, 2008). Based on this, research on a less pollutant fuel was compulsory and has led to the development of bioethanol which allegedly presents a wide range of advantages over fossil fuels.

The main producing countries for transport biofuels are the USA, Brazil and the European Union (EU). Whilst the ethanol in Brazil is mainly produced from sugar cane and in the EU from rapeseed; the production in the United States consists mostly of ethanol from maize (United Nations Environment Programme, 2009). Following experts' prospection the use of maize for ethanol production is expected to continue to grow throughout the years. As a result, the USDA expects that the 33.3 mmt of dried distillers' grain and solubles (DDGS) supplied in 2009-2010 will increase to about 38.6 mmt in 2019-2020. The supply of DDGS in 2009-2010 has doubled the one from 2006-2007 and was four times higher than that of 2003-2004, according to the USDA report on DDGS (Jessen, 2011).

The increased prices of protein sources for animal feeds together with their scarcity have led to the use of alternative protein sources, such as DDGS. However, the inclusion of DDGS in animals' diets must be carefully calculated since toxic compounds such as mycotoxins may be present as contaminants posing a serious threat to animal health, either by their carcinogenic (e.g. aflatoxins, ochratoxin, fumonisins), oestrogenic (zearalenone), neurotoxic (fumonisins), dermatotoxic (deoxynivalenol and T-2 toxin) or by their immunosuppressive (aflatoxins, ochratoxin A, trichothecenes) effects.

During the production of ethanol from maize, mainly the endosperm (82% of the grain) undergoes fermentation and distillation processes. For dry-milled products, the most highly mycotoxin-contaminated fractions are those that contain the whole or the outer portions of the grain (Hazel and Patel, 2004). Based on this knowledge it is widely accepted that the co-products of ethanol production will concentrate by up to three times the previously existing mycotoxins in maize (Wu and Munkvold, 2008).

Some reports are available regarding the fate and occurrence of mycotoxins in the by-products of ethanol production and brewing (Bennett and Richard, 1996; Bothast *et al.*, 1992; Zhang *et al.*, 2009). These reports usually target one country/region or the monitoring of a single mycotoxin and therefore further information may be beneficial. This report aims to increase the level of knowledge on the occurrence of mycotoxins in maize DDGS sourced and used worldwide in animal feeds.

2. Materials and methods

Analytical samples

A total of 409 maize DDGS samples was sourced directly at worldwide animal farms or animal feed production sites from January 2005 until December 2010. 51% of samples were sourced in the USA, 47% in Asia (30% in North Asia, 15% in South-East Asia and 2% in Oceania) and 2% in Europe (1% from Central Europe, 1% from Southern Europe). 1 sample was sourced in Egypt, but its origin was the USA. Five samples were analysed in 2005, 56 in 2006, 100 in 2007, 143 in 2008, 34 in 2009 and 71 in 2010. Sample providers randomly sent their samples for testing and were advised to follow principles of good sampling (Richard, 2000); however, analytical personnel and/or laboratory staff were not involved and therefore were not able to influence any part of this procedure. Approximately 1 kg of sample material was received for analysis. A choice could be made regarding the mycotoxins to be analysed for from the 5 groups of mycotoxins: aflatoxins (reported as aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2) individually and as a sum of these), zearalenone (ZEA), deoxynivalenol (DON), fumonisins B_1 (FB_1) and B_2 (FB_2) (reported individually and as a sum of both) and ochratoxin A (OTA) which explains why the number of analysed mycotoxins in the results is sometimes different depending on the specific mycotoxin. The origin (name and location of submitter) of samples was kept strictly confidential; analytical certificates were submitted only to the originators of samples.

Reagents

Organic solvents, HPLC-grade water, and salts and other chemicals were purchased from Merck (Darmstadt, Germany), Fisher Scientific (Wien, Austria) and Sigma Aldrich (St. Louis, LO, USA). Mycotoxin standards were purchased from Romer Labs Diagnostic GmbH (Tulln, Austria).

Sample preparation and clean-up procedures

Samples were ground and sub-sampled using a Romer® Series II Mill (Romer Labs, Inc., Union, MO, USA). 25 g of each sample was extracted with 100 ml of acetonitrile:water (84:16) in case of AFB_1, AFB_2, AFG_1, AFG_2, OTA, DON and ZEA; with 100 ml of methanol:water (80:20) and 5 g sodium chloride for FB_1 and FB_2. An osterizer blender (Oster, Boca Raton, FL, USA) with 250 ml blender jars (3 min) was used for fumonisins extraction. The extraction of the other toxins was done on a shaker for one hour at 250 rpm speed.

The extract was filtered through a qualitative filter paper 185 mm (Fisherbrand QL100; Fisher Scientific). Clean up for analysis of deoxynivalenol was performed with Romer Labs MycoSep® and MultiSep® clean-up columns; AFB_1, AFB_2, AFG_1, AFG_2, FB_1 and FB_2, and OTA were cleaned up with Romer Starline® immunoaffinity columns. For zearalenone clean-up, both Romer Labs MycoSep® and Starline® immunoaffinity column were used. In brief, sample extract was done by pushing through the MycoSep® column and through a second clean-up column, MultiSep®, in the case of deoxynivalenol. However, for the other toxin tests, a dilution with phosphate buffered saline (PBS) (pH 7.4) was performed and passed through the respective immunoaffinity column at a flow rate of approximately 1 ml/min. After washing with PBS or water, mycotoxins were eluted with methanol. For AFB_1, AFB_2, AFG_1, AFG_2 test, eluate was added to water at a 1:1 ratio, mixed and loaded into the HPLC for direct analysis. For the rest of the toxin tests, the eluate was evaporated until dryness and re-dissolved in mobile phase for HPLC analysis.

High performance liquid chromatography (HPLC)

An HPLC series 1100 from Agilent® technologies (Santa Clara, CA, USA), comprising a vacuum degasser, a binary pump, an auto-sampler, column thermostat, variable

wavelength detector (UV detector) and a fluorescence detector (FLD detector) was used. The UV detector was used for analysis of deoxynivalenol; whereas the FLD detector for analysis of AFB_1, AFB_2, AFG_1, AFG_2, FB_1 and FB_2, OTA and ZEA. For AFB_1, AFB_2, AFG_1, AFG_2 analysis, an electrochemical device (Romer Cell) for post-column derivatisation was involved. FB_1 and FB_2 analysis involved a pre-column derivatisation with the use of derivatising reagents (napthalene 2, 3-dicarboxaldehyde). Table 1 describes the limits of quantification (LOQ), the limits of detection (LOD), the method reference codes and the recovery of the methods applied. For the calculation of LOD and LOQ 10 replicates of blank samples were analysed and standard deviation (SD) was calculated, the LOD being 3 times the SD and the LOQ 10 times the SD.

3. Results and discussion

Table 2 presents the results of analyses performed to the maize DDGS samples. Data are presented by mycotoxin: AFB_1, AFB_2, AFG_1 and AFG_2 and a sum of these (AF), ZEA, DON, FB_1 and FB_2 and a sum of both (FB), and OTA. FB were the most prevalent mycotoxins present in 91% of analysed DDGS samples (average: 1,036; median of positive: 733 µg/kg), followed by ZEA present in 85% of the samples (average: 227; median of positive: 84 µg/kg) and DON, present in 77% of samples (average: 1,755 µg/kg; median of positive: 1,393 µg/kg). OTA and AF were the less prevalent groups of mycotoxins, present in 25% (average: 2 µg/kg; median of positive: 4 µg/kg) and 19% (average: 2 µg/kg; median of positive: 2 µg/kg) of tested DDGS, respectively. The third quartile of positive results indicates that 75% of the samples were above a certain contamination level. Also from Table 2 can be observed that 75% of tested samples had contamination levels above 7 µg/kg AF, 212 µg/kg ZEA, 2,679 µg/kg DON, 1,301 µg/kg FB and 8 µg/kg OTA.

Figure 1 describes in more detail the percentage of samples within several contamination ranges for each mycotoxin. For AF, 81% of analysed samples tested below the LOD, 13% tested between the LOD and 5 µg/kg, 2% of the samples tested within the contamination range 6 to 10 µg/kg, 2% within the range 11-20 µg/kg, and 2% above 21 µg/kg. The maximum contamination level found was 89 µg/kg.

For ZEA, 15% of samples tested below the LOD, 22% were contaminated with less than 50 µg/kg (between 25 and 49 µg/kg), 26% tested positive and within the contamination range 50-100, 15% between 101 and 200 µg/kg, 4% between 201 and 250 µg/kg, 11% between 251 and 500 µg/kg, 5% between 501 and 2,000 µg/kg, 4 samples presented a contamination level between 2,001 and 3,000 µg/kg (1%) and 3 samples presented a contamination higher than 3,000 µg/kg (1%). The maximum contamination level found was 10,374 µg/kg.

Table 1. Limits of quantification, limits of detection, reference code and recovery of methods applied.

Mycotoxins	Limit of quantification (µg/kg)	Limit of detection (µg/kg)	Method reference code	Recovery for maize DDGS (%)
Deoxynivalenol	150	50	LWI-STP-DON-02	70-90
Fumonisin B_1, B_2	200, 200	100, 100	LWI-STP-FUM-01	68-85
Ochratoxin A	1.7	0.5	LWI-STP-OTA-01	80-110
Aflatoxin B_1, B_2, G_1, G_2	1.5, 1.5, 1.5, 1.5	0.5, 0.5, 0.5, 0.5	LWI-STP-AFL-02	80-98
Zearalenone	30	10	LWI-STP-ZON-02	75-95

Table 2. Mycotoxin analysis of dried distillers' grain and solubles[1].

	AFB_1	AFB_2	AFG_1	AFG_2	AF	ZEA	DON	FB_1	FB_2	FB	OTA
Number samples tested	393	393	393	393	393	405	409	390	390	390	173
% positive	18	5	1	0	19	85	77	91	44	91	25
Maximum (µg/kg)	89	19	3	4	89	10,374	24,269	9,042	2,626	9,042	68
Average (µg/kg)	1	0	0	0	2	227	1,755	892	144	1,036	2
1st quartile of positive (µg/kg)	1	0	1	4	1	49	500	389	131	454	2
Median of positive (µg/kg)	2	0	1	4	2	84	1,393	621	211	733	4
3rd quartile of positive (µg/kg)	7	1	2	4	7	212	2,679	1,091	337	1,301	8

[1] Mycotoxin abbreviations used: AFB_1 = aflatoxin B_1; AFB_2 = aflatoxin B_2; AFG_1 = aflatoxin G_1; AFG_2 = aflatoxin G_2; AF = sum of AFB_1, AFB_2, AFG_1 and AFG_2; ZEA = zearalenone; DON = deoxynivalenol; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; FB = sum of FB_1 and FB_2; OTA = ochratoxin A.

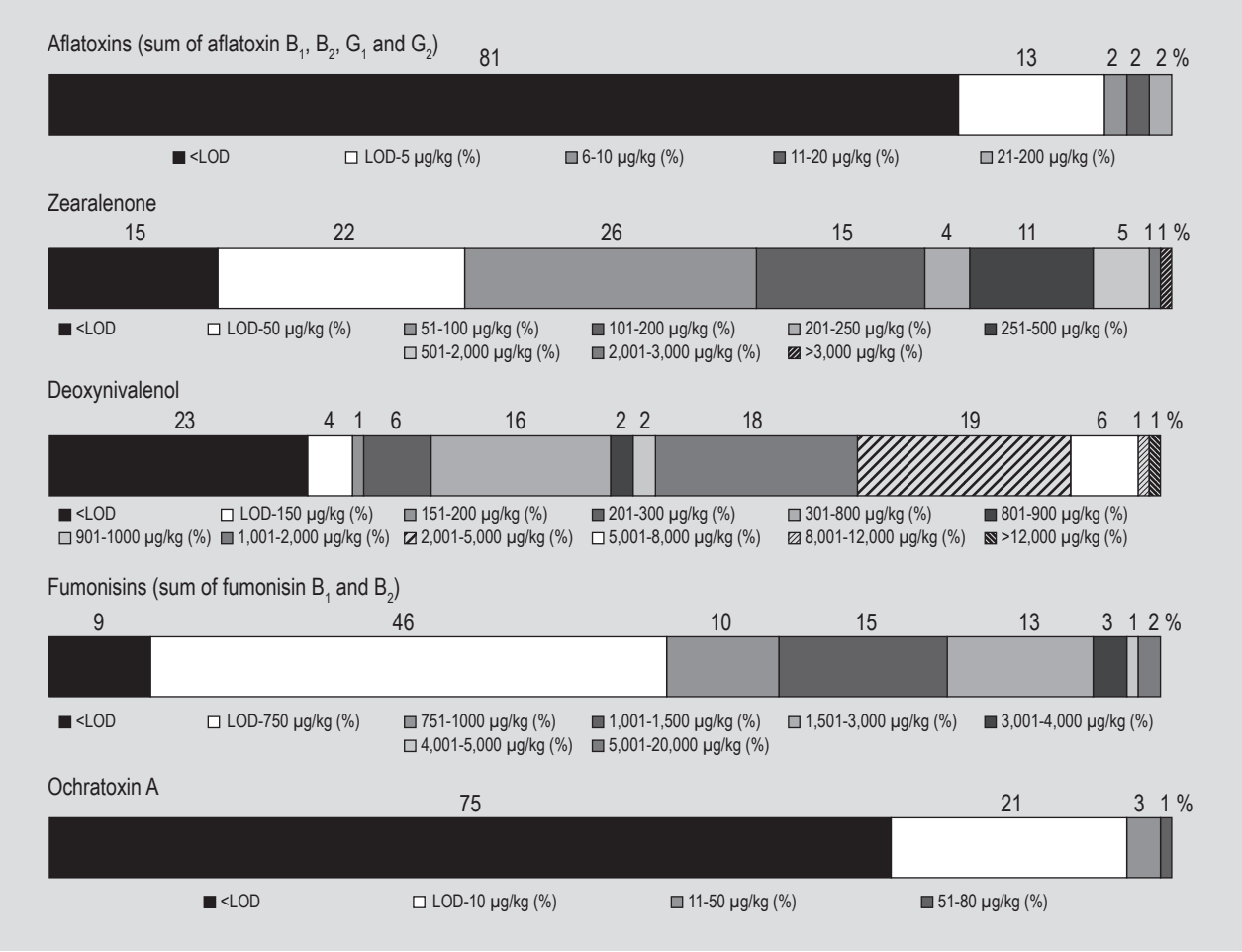

Figure 1. Distribution of aflatoxins (sum of aflatoxin B_1, B_2, G_1 and G_2), zearalenone, deoxynivalenol, fumonisins (sum of fumonisins B_1 and B_2) and ochratoxin A in dried distillers' grain and solubles by contamination range.

In the case of DON, 23% of samples tested below 50 µg/kg (LOD). 4% of samples tested positive and showed contamination levels in the range between 50 and 149 µg/kg, 1% ranged from 150 to 200 µg/kg, 6% from 201 and 300 µg/kg, 16% of samples had contamination levels between 301 and 800 µg/kg, 2% within 801 and 900 µg/kg, 2% within 901 and 1000 µg/kg, 18% had levels between 1,001 and 2,000 µg/kg, 19% between 2,001 and 5,000 µg/kg (79 samples), 6% between 5,001 and 8,000 µg/kg (26 samples), 1% presented levels above 8,000 µg/kg (6 samples) and 5 samples presented levels above 12,000 µg/kg (1%). The maximum contamination level found was 24,269 µg/kg.

In the case of FB, 9% of samples tested below the LOD. 46% tested positive with a contamination range below 750 µg/kg. 10% of FB contaminated samples fell in the range 751-1000 µg/kg, 15% in the range 1,001-1,500 µg/kg, 13% in the range 1,501-3,000 µg/kg, 3% in the range 3,001-4,000 µg/kg, 1% between 4,001-5,000 µg/kg and finally 2% in the range higher than 5,001. The maximum contamination level found was 9,042 ug/kg.

OTA contamination levels observed were quite lower, as 75% of tested samples tested below the LOD. 21% tested positive within the contamination range 0.2 µg/kg (LOD) and 10 µg/kg, 3% presented contamination levels between 11 and 50 µg/kg, and 1% between 51 µg/kg and higher. No contamination levels above 68 µg/kg were detected.

Figure 2 represents the co-occurrence of mycotoxins in the analysed DDGS samples. As depicted, only 2% of the analysed DDGS showed contamination levels below the LOD. 6% of samples had the presence of 1 mycotoxin only and the majority (92% of the samples) was contaminated with 2 or more mycotoxins. The presence of different mycotoxins in feed may lead to synergistic interactions between multiple mycotoxins. Several studies have been carried out with combinations of mycotoxins and the amplification of toxicity in animals has been confirmed for monogastric (Grenier and Oswald, 2011; Speijers and Speijers, 2004) and poligastric animals (D'Mello *et al.*, 1999; Grenier and Oswald, 2011).

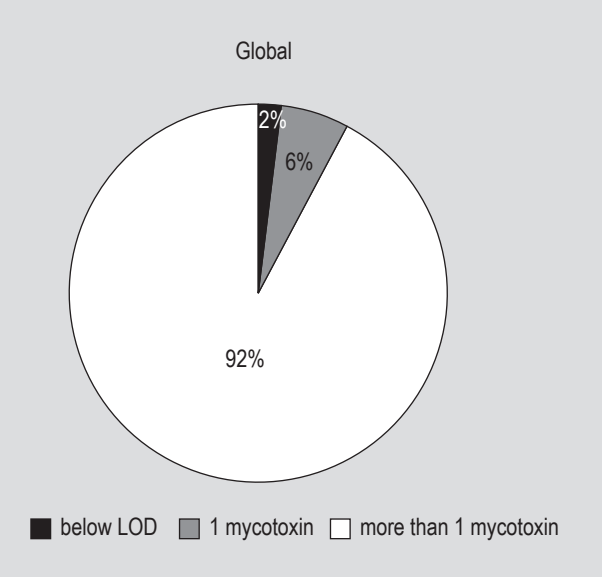

Figure 2. Co-occurrence of mycotoxins in dried distillers' grain and solubles.

Regulations have been adopted in many countries to protect animal and humans from the potential hazardous effects of mycotoxins. The US Food and Drug Administration (FDA) has developed action levels for aflatoxins, guidance levels for fumonisins and advisory levels for DON. As for ZEA and OTA no action, guidance or advisory levels were developed so far (FDA, 2006). Likewise, Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 sets up 20 µg/kg as the maximum content of aflatoxin B_1 allowed for all feed materials intended for animal feed (EC, 2002). Recommendations on the presence of other mycotoxins such as DON, ZEA, OTA, T-2 and HT-2 toxin are given in Commission Recommendation of 17 August 2006 for products intended for animal feeding. This recommendation sets up 12,000 µg/kg DON, 3,000 µg/kg ZEA, 250 µg/kg OTA and 60,000 µg/kg FB as guidance values for maize by-products (EC, 2006).

Results of this survey differ from those of Zhang *et al.* (2009) where none of the tested DDGS samples had AF or DON contamination above the FDA threshold levels for use in animal feed. FDA's minimum action level for aflatoxins is 20 µg/kg for feed ingredients used in immature animals, dairy cattle, or intended use is unknown. 8 DDGS samples (2%) analysed in this survey showed AF levels above that threshold, thus exceeding FDA's limits. Only 7 DDGS samples exceeded the European thresholds of 20 µg/kg AFB_1. The difference relies on the fact that the latter accounts only for AFB_1 and not for the sum of aflatoxins. For DON, 5 mg/kg (5,000 µg/kg) is the FDA's advisory level for feed ingredients used in swine diets (not to exceed 20% of the total diet or 40% in case of other animal species). From the 409 samples analysed for DON in this survey 37 (8%) exceeded this limit. Five of the analysed samples exceeded the 12,000 µg/kg threshold imposed by the Commission Recommendation. On the other hand, only 2% (8 samples) of the DDGS samples analysed for FB exceeded the 5 mg/kg guidance level (limit for feed ingredients used for equids and rabbits at no more than 20% of the diet). The occurrence of FB in this survey was lower than that of the abovementioned report. Three samples (1%) had ZEA levels above the Commission Recommendation of 3,000 µg/kg. No samples exceeded the threshold levels for FB and OTA.

4. Conclusions

Although DDGS may be seen as a practical solution for animal producers, enabling them to counteract the rising prices of feedstuffs and feed, the widespread carefree use of these products is still not possible. The increasing awareness of the purchasers to this reality may also have an impact on DDGS industrials as animal producers may seek for assurance regarding the safety of the purchased products. Monitoring the mycotoxin content of DDGS prior to its inclusion in animals' diets is crucial to avoid the exposure of animals to the negative effects of mycotoxins. This work demonstrated the high prevalence of mycotoxins, especially FB, ZEA and DON within the 409 maize DDGS samples analysed from 2005 to 2010.

References

Bennett, G.A. and Richard, J.L., 1996. Influence of processing on *Fusarium* mycotoxins in contaminated grains. Food Technology 50: 235-238.

Bothast, R.J., Bennett, G.A., Vancauwenberge, J.E. and Richard, J.L., 1992. Fate of fumonisin B_1 in naturally contaminated corn during ethanol fermentation. Applied and Environmental Microbiology 58: 233-236.

D'Mello, J.P.F., Placinta, C.M. and MacDonald, A.M.C., 1999. *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity. Animal Feed Science and Technology 80: 183-205.

European Commission (EC), 2002. Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Official Journal of the European Union L 140: 10-22.

European Commission (EC), 2006. Commission Recommendation (2006/576/EC) of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Official Journal of the European Union L 229: 7-9.

Food and Drug Administration (FDA), 2006. Mycotoxins in feeds: CVM's perspective. Available at: http://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/Contaminants/ucm050970.htm.

Grenier, B. and Oswald, I.P., 2011. Mycotoxin co-contamination of food and feed: meta-analysis of publications describing toxicological interactions. World Mycotoxin Journal 4: 285-313.

Hazel, C.M. and Patel, S., 2004. Influence of processing on trichothecene levels. Toxicology Letters 153: 51-59.

Jessen, J., 2011. Hot destinations for DDGS exports: China, Mexico, Canada. Available at: http://www.ethanolproducer.com/articles/7486/hot-destinations-for-ddgs-exports-china-mexico-canada.

Richard, J., 2000. Sampling and sample preparation for mycotoxin analysis. Romer Labs guide to mycotoxins, Vol. 2. Romer Labs., Union, MO, USA.

Speijers, G.J.A. and Speijers, M.H.M., 2004. Combined toxic effects of mycotoxins. Toxicology Letters 153: 91-98.

United Nations Environment Programme, 2009. Towards sustainable production and use of resources: assessing biofuels. Available at: http://www.unep.fr/scp/rpanel/pdf/Assessing_Biofuels_Full_Report.pdf.

United Nations Framework Convention on Climate Change (UNFCCC), 1994. Article 2. Available at: http://unfccc.int/essential_background/convention/background/items/1353.php.

US Environmental Protection Agency (US EPA), 2008. U.S. EPA's 2008 report on the environment (final report). EPA/600/R-07/045F (NTIS PB2008-112484). US EPA, Washington, DC, USA.

Wu, F. and Munkvold, G.P., 2008. Mycotoxins in ethanol co-products: modeling economic impacts on the livestock industry and management strategies. Journal of Agricultural and Food Chemistry 56: 3900-3911.

Zhang, Y., Caupert, J., Imerman, P.M., Richard, J.L. and Shurson, G.C., 2009. The occurrence and concentration of mycotoxins in U.S. distillers dried grains with soluble. Journal of Agricultural and Food Chemistry 57: 9828-9837.