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REVIEW

Mycotoxins in Ethanol Co-products: Modeling Economic Impacts on the Livestock Industry and Management Strategies

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The rapidly expanding U.S. ethanol industry is generating a growing supply of co-products, mostly in the form of dried distillers' grain and solubles (DDGS) or wet distillers' grains (WDG). In the United States, 90% of the co-products of maize-based ethanol are fed to livestock. An unintended consequence is that animals are likely to be fed higher levels of mycotoxins, which are concentrated up to three times in DDGS compared to grain. The model developed in this study estimates current losses to the swine industry from weight gain reduction due to fumonisins in added DDGS at $9 million ($2–18 million) annually. If there is complete market penetration of DDGS in swine feed with 20% DDGS inclusion in swine feed and fumonisins are not controlled, losses may increase to $147 million ($29–293 million) annually. These values represent only those losses attributable to one mycotoxin on one adverse outcome on one species. The total loss due to mycotoxins in DDGS could be significantly higher due to additive or multiplicative effects of multiple mycotoxins on animal health. If mycotoxin surveillance is implemented by ethanol producers, losses are shifted among multiple stakeholders. Solutions to this problem include methods to reduce mycotoxin contamination in both pre- and postharvest maize.

KEYWORDS: Mycotoxins; ethanol; co-products; distillers' grains; livestock feed; animal health risk; economic impacts

INTRODUCTION

Ethanol has received increasing attention in the United States in recent years as a potentially cost-effective renewable energy source. In the United States, ethanol is currently almost entirely produced from maize (*1*). Hence, maize prices have increased, and maize planting acreage in the United States in 2007 rose to its highest level in 63 years (*2*).

One potential health risk of ethanol production from maize concerns mycotoxins in the ethanol co-products, or dried distillers' grains and solubles (DDGS). Mycotoxins are defined as toxic or carcinogenic chemicals that are secondary metabolites of fungi that colonize crops. When ingested, these mycotoxins can cause a number of adverse health effects in animals and humans. The majority of current ethanol production and planned ethanol expansion utilizes a dry-grind process for initial milling. The fermentation and distillation processes by which dry-grind ethanol is produced concentrate the previously existing mycotoxin levels in maize up to three times in the co-products (*3–6*). These coproducts, with higher mycotoxin levels than the original grain, are then marketed for inclusion as a livestock feed component.

With elevated maize prices, swine, poultry, and dairy cattle producers may find it increasingly desirable in the short term to include more DDGS in their animals' diets. In the long term, however, the adverse health effects of elevated mycotoxin levels in DDGS could cause nontrivial economic damage to the livestock industry, especially for more sensitive species such as swine. This paper is the first review of the potential impact to animal health of mycotoxins in DDGS and of the potential economic impacts. We first describe the impact of ethanol production on maize planting and prices. Next we list agriculturally important mycotoxins in maize and their animal health impacts. Studies on the fate of mycotoxins in grain during ethanol production or other fermentation processes, leading to mycotoxin accumulation in the finished co-product, are sum-

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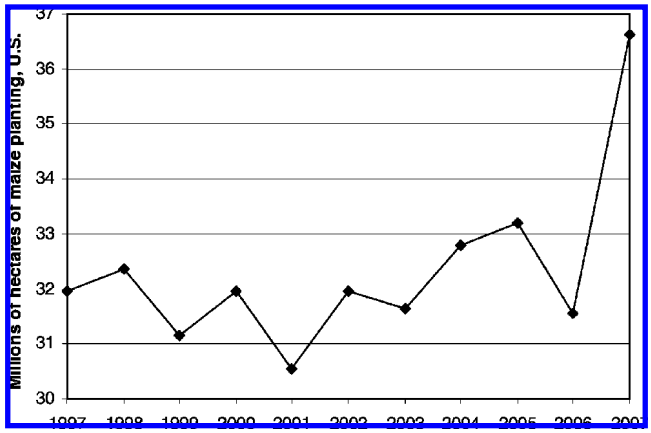

Figure 1. Maize planting in millions of hectares in the United States, 1997−2007 (*2*).

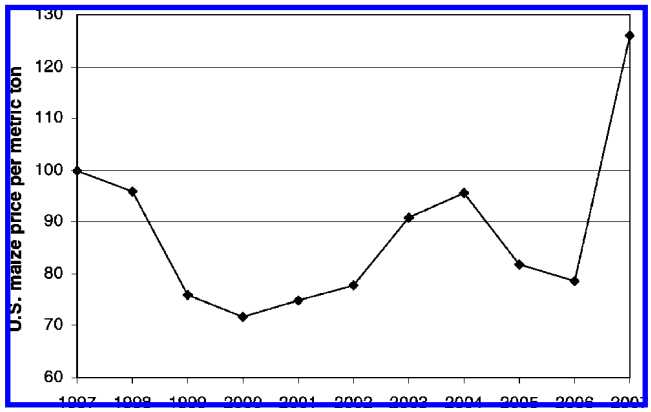

Figure 2. Maize price per metric ton, USD, 1998–2007 (*7*).

marized. Possible health effects on livestock from the inclusion of DDGS in diets are discussed, and economic impacts to the swine industry from one health effect—reduced weight gain—caused by mycotoxin consumption are estimated. Finally, potential solutions are described.

MAIZE-BASED ETHANOL AND CO-PRODUCTS

In 2007, U.S. farmers planted 36.6 million hectares of maize, the largest area devoted to maize since 1944 and 4.9 million hectares more than in 2006 (*2*). At the same time, maize prices were significantly higher last year—$3.20 per bushel ($125.70 per metric ton)—than in previous years. **Figure 1** shows land area planted to maize in the United States over the past 10 years, wheereas **Figure 2** (price of maize per bushel) shows the recent increase in maize prices that reflects this demand. It is unusual for both supply and price of a good to increase simultaneously at such a dramatic level. In this case, the rise in both supply and price is due primarily to the demand for maize-based ethanol. Because maize prices have risen as a result of ethanol demand, livestock farmers are facing a challenge in affordably feeding their animals. One way to deal with the rising price of feed is to include DDGS, which provides a source of protein, along with other feedstuffs in animal diets. The proportion of DDGS in animal diets is expected to increase as it becomes increasingly available as a co-product of ethanol.

The majority of the maize produced in the United States is used for animal feed. However, the proportions of total maize production used for different purposes have changed in the past decade. In 2000, animal feed and ethanol made up 58 and 6% of maize use, respectively (*8*). In 2006, however, the respective percentages were 54.5 and 14.3% (*9*). Currently, ethanol plants

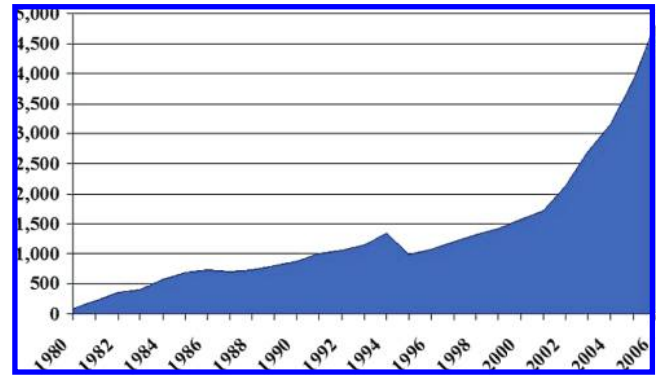

Figure 3. U.S. ethanol production, millions of gallons, 1980–2006 (*9*).

produce 10.2 L of ethanol and 8.2 kg of distillers' grain from each bushel (25.5 kg) of maize (*9*).

Figure 3 shows how ethanol production in the United States increased steadily between 1980 and 2000 and then accelerated rapidly from 2000 through 2006. Indeed, the National Corn Growers Association (NCGA) has presented a vision termed "15 × 15 × 15"; the intent is to produce 15 billion bushels of maize and 15 billion gallons of ethanol by the year 2015 (*10*). To meet this goal, ethanol production must increase to three times the current production levels (see **Figure 3**), and maize production must increase by almost 5 billion bushels from the current production of 10.5 billion (*2*). (There are 39.37 bushels of maize in 1 t.)

When maize is fermented to form ethanol, two main co-products are formed. One is coarse unfermented distillers' grain (DG) residue (in wet form, approximately 50% moisture; or dried to approximately 10% moisture); the other is the liquid fraction or thin stillage, composed of yeast, fine grain particles, and soluble nutrients, condensed to form condensed corn distillers' solubles (CCDS). When these co-products are combined and dried, the product is referred to as DDGS. Wet distillers' grains (WDG) are desirable from a cost-saving standpoint because drying DG is energy-intensive. However, WDG are expensive to transport and have limited storage capability. The majority of ethanol co-products are fed to livestock in the form of DDGS, although the use of WDG is increasing. These ethanol co-products are sold directly to livestock producers, commodity brokers, and manufacturing facilities (*6*). Approximately 90% of this material is eventually utilized as an animal feed component. The ratio of DDGS to other materials in the animals' diet plays an important role in the impact of any mycotoxins in the feed on animal health and, ultimately, livestock industry losses.

MYCOTOXINS: ANIMAL HEALTH EFFECTS AND CONCENTRATION FACTOR IN DISTILLERS' GRAINS

Five important mycotoxins in maize are fumonisin, aflatoxin, deoxynivalenol (DON, or vomitoxin), zearalenone, and ochratoxin A. Leung et al. (*11*) provide structural properties of these mycotoxins, as well as other mycotoxins that may pose risks in maize. All of these mycotoxins produce a variety of toxic or carcinogenic effects in humans and animals. **Table 1** lists the animal health effects associated with each of the five primary mycotoxins.

Fumonisins are a recently discovered class of mycotoxins produced by the fungi *Fusarium verticillioides* (formerly *F. moniliforme*), *Fusarium proliferatum*, and some related species (*47*). *F. verticillioides* is an almost-universal inhabitant of maize. The first report implicating fumonisins in human disease was

Table 1. Five Agriculturally Important Mycotoxins in Maize and Animal Health Effects

mycotoxin	animal health effects	refs
fumonisin	disrupts sphingolipid metabolism in multiple species horses: equine leukoencephalomalacia rodents: liver and kidney cancer, alterations in kidney function poultry: cytotoxicity in turkey lymphocytes swine: porcine pulmonary edema, reduced weight gain, immunosuppression, cardiovascular dysfunction	*12–21*
aflatoxin	causes DNA modification and cell deregulation, leading to liver toxicity and cancer in many species cattle: tachycardia, death, aflatoxin M1 secreted in milk poultry: impaired productivity, decreased egg production, brittle eggshells swine: weight loss, anorexia, ataxia, coma, death	*22–30*
deoxynivalenol	inhibits protein and DNA synthesis in multiple species; causes immunosuppression swine: diminished feed consumption, lower weight gain, vomiting poultry and cattle: same effects at higher concentrations	*31–38*
zearalenone	estrogenic effects, swelling vulva and mammaries, pseudopregnancy in swine; similar effects at higher levels in poultry and cattle	*39–41*
ochratoxin A	disrupts phenylalanine metabolism, damages kidneys in multiple species; causes immunosuppression and increases cancer risk poultry: nephrotoxicity, decreased feeding, ataxia, death swine: changes in renal function, altered urine excretion	*42–46*

in connection with high human esophageal cancer rates in Transkei, South Africa, in 1988; the following year, interest in these mycotoxins increased dramatically after unusually high horse and swine death rates in the United States were linked to contaminated feed (*48*). Fumonisin causes toxic effects through its inhibition of ceramide synthase, an enzyme that is necessary for sphingolipid metabolism. In humans, fumonisin has been implicated in neural tube defects in the fetus (*49*). Elevated levels of fumonisin in animal feed cause diseases such as equine leukoencephalomalacia (ELEM) in horses and porcine pulmonary edema (PPE) and liver damage in swine (*12–15*). In addition, fumonisin has been associated with reduced weight gain in swine (*19*). Fumonisin has been shown to cause liver and kidney cancer in rats and liver cancer in mice (*13*), as well as alterations in kidney function (*17, 18*). It is also cytotoxic to turkey lymphocytes (*16*).

Aflatoxins are produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Four major aflatoxins (B_1, B_2, G_1, and G_2) and two metabolic products (M_1 and M_2) are important contaminants of foods and feeds (*41*). Aside from maize, aflatoxin is also a common contaminant of peanuts, pistachios, almonds, hazelnuts, and cottonseed. Aflatoxin B_1, the most toxic of the aflatoxins, causes a variety of adverse effects in different animal species through DNA modification and cell deregulation. The most prominent effects are liver damage, gastrointestinal dysfunction, and immunosuppression (*22, 24, 29*). In poultry, aflatoxin causes liver damage, impaired productivity and reproductive efficiency, decreased egg production in hens, inferior egg-shell quality, inferior carcass quality, and increased susceptibility to disease (*26, 31*). Swine that consume aflatoxin experience weight loss, anorexia, ataxia, tremore, coma, and death (*29*). In cattle, the primary clinical signs are reduced weight gain as well as liver and kidney damage. Milk production is reduced, and aflatoxin M1 is excreted in the milk (*23, 25, 27*). Aflatoxins have been responsible for human fatalities, as recently as 2004 (*50*).

Deoxynivalenol is produced by the fungus *Fusarium graminearum* and the related species *Fusarium culmorum* in cooler climates. It causes *Fusarium* head blight in wheat and barley, and *Gibberella* or pink ear rot in maize (*51*). DON is an inhibitor of protein synthesis and causes human and animal effects ranging from feed refusal, vomiting, and nausea to immunosuppression and loss of productivity. Among farm animals, swine are typically the most sensitive to DON, whereas poultry and cattle have higher tolerance (*34*). In swine, feed refusal and decreased weight gain are the principal clinical effects (*32, 35, 36*); at higher DON exposures, vomiting and other clinical signs of abdominal distress appear (*31*). DON and other trichothecene mycotoxins—T-2 toxin, nivalenol, and diacetoxyscirpenol (also produced by *Fusarium* spp.)—are important immunosuppressors in animals. Acute exposure to trichothecenes can result in severe damage to the bone marrow, lymph nodes, spleen, thymus, and intestinal mucosa (*37, 38*).

Zearalenone, like DON, is produced by *F. graminearum*. Zearalenone is sometimes referred to as a mycoestrogen, as it causes estrogenic responses and vulvovaginitis in swine (*39*). These swine health effects are observed when the concentration of zearalenone in maize is >0.25 mg/kg (*52*). Also, the mycoestrogen can be transmitted to piglets in sow's milk, causing estrogenism in the piglets (*41*). At higher concentrations, zearalenone causes similar effects in poultry and cattle (*40*).

Ochratoxin A (OTA) is the major mycotoxin in a group of structurally similar metabolites produced by *Aspergillus ochraceus* and *Penicillium verrucosum* in grains (*41*). It disrupts phenylalanine metabolism, causing kidney damage in multiple species. In addition, it leads to immunosuppression (*45*) and increased cancer risk (*44*). In swine, OTA causes changes in renal function and altered urine excretion (*42*). In poultry, it causes reduced growth, decreased feed conversion, reduced egg production, brittle egg shells, and mortality (*43, 46*).

Mycotoxin Concentration in Distillers' Grains. Mycotoxin risk associated with ethanol co-products is dependent on the fate of mycotoxins present in the original grain. Several studies have reported on mycotoxin fate during ethanol production or brewing (*3, 4, 53–58*), using either naturally contaminated grain or grain artificially contaminated by the addition of known quantities of pure mycotoxins. It is generally believed that during fermentation and distillation of maize to produce ethanol, there is very little degradation of mycotoxins. In one study on aflatoxins in maize and other grains (*59*) and another on ochratoxin A in barley (*56*), significant degradation of mycotoxins was reported during fermentation, but that result conflicts with the majority of published reports. Mycotoxins are not found in distilled ethanol, and most studies show that the original mycotoxin content remains largely intact in the other fractions, including WDG and other fractions usually combined into DDGS, or other livestock feed co-products (*4*). Because these fractions represent a smaller mass than the original grain, the concentration of mycotoxins is typically higher in DDGS than in the original grain. Indeed, an accepted "rule of thumb" is that the mycotoxin concentration in DDGS is *three times* that in the original maize (*6*). This assumption may be based partially

Table 2. Published Reports of Mycotoxin Recovery from Maize Fermentation Fractions

mycotoxin	initial level (ng/g)	contamination method	recovery (%)	distillers' grains (ng/g)	concn factor (DDG or DDGS)	distillers' solubles (ng/g)	ref
aflatoxins	100	artificial	80.5	264[a]	2.64	NR	*5*
	204	artificial	97.4	652[a]	3.20	NR	*5*
	343	artificial	93.4	1053[a]	3.07	NR	*5*
	772	artificial	93.0	2362[a]	3.06	NR	*5*
aflatoxins	195	natural	NR[c]	500[b]	2.56	189	*55*
	5386	natural	NR	9868[b]	1.83	2953	*55*
aflatoxins	617	natural	NR	952	1.54	NR	*58*
	543	natural	NR	1107	2.04	NR	*58*
fumonisins	0	natural	85.0	4000–5000	NA	0–200	*4*
	15000	natural	85.0	19200–25300	1.28–1.69	1300–1700	*4*
	36000	natural	85.0	48500–65000	1.35–1.81	4900–5800	*4*
zearalenone	8000	natural	NR	18000–20000	2.25–2.50	10000–12000	*53*
	33500	natural	NR	50000–62000	1.49–1.85	14000	*53*

[a] Calculated for distillers' grains and solubles combined, assuming 56 lb/bu corn and 18 lb of DDGS/bu. [b] Calculated for dried distillers' grains, assuming 50% MC for wet distillers' grains and 10% for DDGS. [c] Not reported or not estimable from available information.

on empirical observations. The dry mass of DDGS is approximately one-third that of the original grain; if one assumes mycotoxin degradation is negligible, a 3 times higher concentration in DDGS versus grain would be expected.

The results of previous studies (*5, 53, 60*; Dr. Arthur Schaafsma, University of Guelph, personal communication) support the occurrence of 3 times higher concentrations in DDGS. Data are available for aflatoxins, deoxynivalenol, fumonisins, and zearalenone. In some cases the increase is <3 times or cannot be calculated (**Table 2**). Mycotoxin recovery is reported differently among the published studies, and it is not always possible to estimate the concentration factor. In some reports, mycotoxin recovery is not reported as a concentration (e.g., ng/g), but as a raw amount (e.g., total μg) and is reported separately for various fractions that are combined to produce DDGS. These fractions differ in moisture content (usually not reported), and the final mycotoxin concentration in DDGS also would vary according to proportions of DG and solubles in the final product. These issues prevent a quantitative comparison of mycotoxin concentrations in grain versus final co-products for some of the reports. It is consistent, however, that the majority of recoverable mycotoxin is found in the DG fraction, and the concentration of these toxins is elevated in comparison to the concentration in the initial grain. In at least one example, fumonisins that were not detectable in the original grain were detected at 4000−5000 ng/g in DG (*4*). This result may have been due to sampling error in the original grain analysis or to a concentration of fumonisins from below the detection limit in grain to a measurable level in DG. The consistent elevation of mycotoxin levels in co-products magnifies the importance of mycotoxins in maize grain used for ethanol production.

MEETING ANIMAL FEED STANDARDS FOR MYCOTOXINS

As distillers' grains makes up an increasing proportion of animal feed, it is important to consider how much DDGS can be included in animal diets and still ensure that the overall feed meets the U.S. Food and Drug Administration's (FDA) guidelines and action levels on mycotoxins. Indeed, for this reason, it is important to ensure that the maize entering ethanol facilities has acceptable mycotoxin levels. In addition, DDGS is now a significant export product for the United States, with about 1.2 million metric tons exported in 2006 (*61*). The European Union (EU) is a major export market for DDGS, and the EU has mycotoxin standards that are considerably more stringent than those in the United States. Application of stringent mycotoxin limits on DDGS imports to the EU or other destinations could lead to additional economic effects for the U.S. ethanol industry.

The FDA's guidelines for industry and action levels on mycotoxins in feed are based on risk assessment in a variety of species, examining adverse health effects at different mycotoxin concentrations. **Table 3** lists the FDA standards for different species for each regulated mycotoxin.

Many of these standards are based on assumptions about the percentage of an animal's diet that is composed of maize. The inclusion of DDGS in the diet, combined with maize and other feedstuffs such as soybean meal, may alter these assumptions and possibly require revised mycotoxin standards that account for levels in ethanol co-products.

How Much DDGS Can Be Added to Feed To Meet Guidelines? No animal feed can consist of 100% DDGS. Limiting factors for the maximum acceptable proportion of DDGS in livestock diets include the high fiber and fat content, lack of key amino acids, lack of rumen degradable protein (*62*), sulfur content (*63*), and potentially dangerously high mycotoxin concentrations. DDGS is, however, a protein-rich source that is used to replace portions of both maize and soybean meal, with the addition of key amino acids. On the basis of factors other than mycotoxins, it is recommended that DDGS can comprise up to 30% of dairy cattle feed (*62*), up to 50% of feed for older heifers (*64*), 25% of nursery swine feed, 20% of grow−finish and lactating swine feed, and 50% of gestating sow and boar feed (*65*). Poultry can be fed up to 20% DDGS with no difference in performance compared with controls (*66*).

ECONOMIC MODEL OF IMPACTS ON LIVESTOCK INDUSTRY

The influence diagram in **Figure 4** shows the relationship between the various factors that affect mycotoxin concentrations in DDGS and animal feed, animal health effects, and our objective function: *the economic impact on the livestock industry*. The variables in the model are defined as follows: $M(0)$, mycotoxin concentration in original grain; C, mycotoxin concentration factor in ethanol processing; M(DDGS), mycotoxin concentration in distillers grains; P(DDGS), proportion of distillers grain in animal feed; M(feed), mycotoxin concentration in animal feed; P(ill), proportion of animal units affected

Table 3. FDA Standards on Mycotoxins in Animal Feed[a]

mycotoxin	FDA standard for animal feed
total fumonisin (FB_1 + FB_2 + FB_3)[b]	5 mg/kg for equids and rabbits (<20% of diet[c]) 20 mg/kg for swine and catfish (<50% of diet) 30 mg/kg for breeding ruminants, breeding poultry and breeding mink (<50% of diet) 60 mg/kg for ruminants >3 months old raised for slaughter and mink raised for pelt production (<50% of diet) 100 pm for poultry raised for slaughter (<50% of diet) 10 mg/kg for all other species or classes of livestock and pets (<50% of diet)
total aflatoxin (AFB_1 + AFB_2 + AFG_1 + AFG_2)[d]	300 μg/kg for maize and peanut products intended for finishing (i.e., feedlot) beef cattle 300 μg/kg for cottonseed meal intended for beef cattle, swine, or poultry (regardless of age or breeding status) 200 μg/kg for maize or peanut products intended for finishing swine of 100 lb or greater 100 μg/kg for maize and peanut products intended for breeding beef cattle, breeding swine, or mature poultry 20 μg/kg for maize, peanut products, and other animal feeds and feed ingredients, but excluding cottonseed meal, intended for immature animals 20 μg/kg for maize, peanut products, cottonseed meal, and other animal feeds and feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known
deoxynivalenol[e]	10 mg/kg for ruminating beef and feedlot cattle> 4 months, chickens (<50% of diet) 5 mg/kg for swine (<20% diet) 5 mg/kg for all other animals (<40% diet)

[a] The standards for total fumonisins and DON are guidelines for industry, whetrsd those for total aflatoxins are action levels. [b] Source: http://www.cfsan.fda.gov/~dms/fumongu2.html. [c] Assuming the feed item (e.g., maize) makes up no more than this proportion of the animal's diet. [d] Source: http://www.cfsan.fda.gov/~lrd/fdaact.html. [e] Source: http://www.cfsan.fda.gov/~dms/graingui.html.

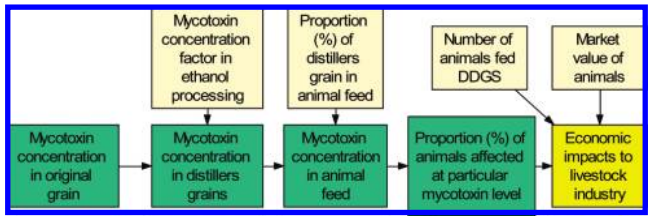

Figure 4. Factors influencing economic impacts on livestock industry of mycotoxins in distillers' grain.

at particular mycotoxin level; N, number of animals affected; and V, market value per animal unit affected.

At present, the mycotoxin concentration in the distillers' grain is purely a function of the mycotoxin concentration in the original maize; hence

$$M(\text{DDGS}) = M(0) \times C \quad (1)$$

Assuming that the animal feed consists of maize, soybean meal, and DDGS, with added nutrients as necessary, the mycotoxin concentration in the final animal feed is a function of the mycotoxin concentrations in both the maize and the DDGS, multiplied by the relative amounts of maize and DDGS in the feed. We assume that the proportion of feed that is soybean meal, P(soy), has negligible mycotoxin levels (*67*):

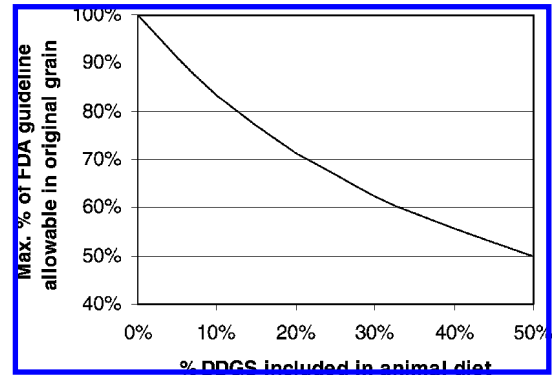

Figure 5. Maximum percentage of FDA guideline allowable in original maize, at different proportions of DDGS included in the animal diet.

$$M(\text{feed}) = M(\text{DDGS}) \times P(\text{DDGS}) + M(0) \times [1 - P(\text{DDGS}) - P(\text{soy})] \quad (2)$$

The animal unit in question is either the absolute number of animals or the weight of the animals (depending on which unit is traded). For example, the swine market in the United States is based on weight (*68*). Hence, eq 3

$$\text{P(ill)} = f[M(\text{feed})] \quad (3)$$

has the dimension either of number of animals affected or total weight lost as a result of mycotoxin consumption, where f denotes a function that represents the dose–response relationship between an animal effect and the proportion of mycotoxin in the diet. Functional forms for this dose–response curve are derived from the literature on the impact of a particular mycotoxin on an animal species. Finally

$$\text{economic impact on livestock industry} = P(\text{ill}) \times N \times V \quad (4)$$

This model was developed in Analytica (www.lumina.com), a software modeling tool that allows input of quantitative values for each variable. These variables are represented by probability density functions to reflect the uncertainty and variability associated with each variable, the parameters of which are estimated from the literature.

First, we were interested in ascertaining the maximum safe mycotoxin level in the original maize if it is to be combined with DDGS and to be fed to animals, in order that the entire feed can meet FDA standards. We assumed that the DDGS displaces amounts of maize and mycotoxin-free soybean meal proportional to their original amounts in the diet (with the addition of key amino acids), that the remainder of the maize in the feed has a particular mycotoxin concentration, and that the mycotoxin concentration in DDGS is three times that of the original maize. **Figure 5** represents our calculations of the percentage of the FDA mycotoxin standard that the original maize should meet, at different concentrations of DDGS in the feed. However, if the original maize used for feed has a decreased mycotoxin level compared to the maize used to produce the DDGS, then the levels of mycotoxins in the DDGS maize could be much higher and still result in an overall "safe" level in animal feed.

For example, if DDGS comprises 30% of the diet, the maize must have at most 62.5% of the FDA mycotoxin guideline for feed. On the other hand, if DDGS comprises 50% of the diet, then the original maize must have no more than 50% of the FDA mycotoxin guideline for feed in order to meet the final feed standard. Including 50% DDGS results in the overall mycotoxin concentration being twice as high as feed containing

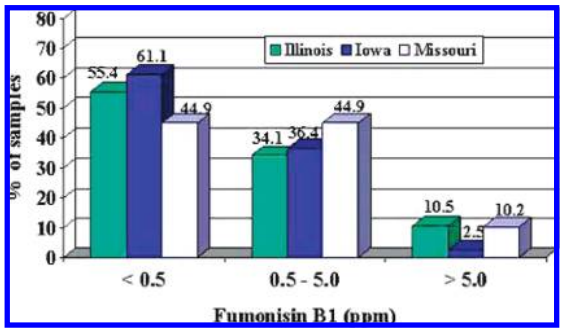

Figure 6. Concentrations of fumonisin B1 in maize samples in three maize-producing states, mid-1990s (*69*).

no DDGS, assuming that the mycotoxin level of the original maize used to produce the DDGS is the same as the mycotoxin level of the maize used to produce the final feed ration. This would indeed be the case for gestating sow and boar feed, if the above recommendations were followed to the maximum level (*64, 65*).

These results may pose problems in that, even if DDGS were not included in the diet, a sizable proportion of the maize produced in the United States already has mycotoxin levels that reach or exceed the FDA standards for animal feed. **Figure 6** shows fumonisin B_1 concentrations in maize samples from three major maize-producing states in the mid-1990s. In some states, >10% of the maize samples had concentrations exceeding 5 mg/kg. An even higher percentage than this would thus have total fumonisin concentrations ($B_1 + B_2 + B_3$) exceeding 5 mg/kg, the standard for horse feed. A 2005 maize survey collected by the Illinois Department of Agriculture showed that 23 of 211 samples statewide (11%) had fumonisin B_1 levels above 5 mg/kg, and 4 (2%) had levels higher than 10 mg/kg (Paul Bertels, National Corn Growers Association, personal communication).

Estimated Losses to the Swine Industry from Reduced Weight Gain. We applied this model to one specific case study: the impact of fumonisin on weight gain reduction in swine. (For a more comprehensive impact estimate of mycotoxins in DDGS, multiple mycotoxins and multiple health effects across multiple species should be calculated.) A concentration factor of 3 was assumed for the concentration of fumonisin in DDGS, and a log-normal probability density function was fitted to fumonisin concentrations in maize on the basis of Munkvold's (*69*) data.

For the dose–response relationship, data from Rotter et al. (*19*) were used as an *upper bound* for possible effects of FB_1 in swine, as no other studies have replicated these results. [Other studies have indeed shown reduced weight gain in swine fed FB_1 alone or in combination with other mycotoxins (*70, 71*); however, the FB_1 doses tested in these studies were higher than those tested in Rotter et al. (*19*).] This study examined the impact on overall weight gain in swine fed at different fumonisin B_1 concentrations over 8 weeks. At 0.1 mg/kg FB_1, swine experienced erratic growth patterns but no significant weight gain difference compared with the control group (no FB_1 in diet). At 1 mg/kg FB_1, swine weight was on average 8% lower than the control group's. At 10 mg/kg FB_1, swine weight was 11% lower than the control group's, and there was detectable liver tissue damage. Aside from a lack of corroborating results from other studies, this study's limitations include finding weight gain reduction only in male swine, lack of reporting variability in weekly weight gains, and a relatively high *P* value (0.059) for weight gain reduction in FB_1 treatment groups. Hence, we assumed that a reasonable mean weight gain reduction in swine from FB_1 consumption was *half* these results and that a lower bound would be *10%* of these results.

Table 4. Weight Gain Reduction Losses to Swine Industry Due to Fumonisin in DDGS[a]

DDGS inclusion in swine diet (%)	market penetration of DDGS in swine feed (%)	expected additional annual loss to swine industry through reduced weight gain ($)
5	12	4 million (1–9 million)
	25	9 million (2–18 million)
	50	18 million (4–37 million)
	100	37 million (7–73 million)
10	12	9 million (2–18 million)
	25	18 million (4–37 million)
	50	37 million (7–73 million)
	100	74 million (15–147 million)
20	12	18 million (4–37 million)
	25	37 million (7–73 million)
	50	74 million (15–147 million)
	100	147 million (29–293 million)

[a] Values are expressed in means, with lower and upper confidence intervals in parentheses.

We estimated the *difference* in weight gain reduction caused by the introduction of DDGS into the diet, compared with the state in which swine feed contained no DDGS. In the latter case, there still would be some weight gain reduction expected in swine because of maize's naturally occurring fumonisin levels. We assumed that DDGS displaced both maize and soybean meal proportional to the original amounts in the diet and that the total value of hog production in the United States was $14.1 billion [the 2006 value of the swine industry; (*68*)]. We did a sensitivity analysis on the effect of including DDGS at 5, 10, and 20% of swine feed.

Table 4 summarizes our results. The annual loss to the swine industry ultimately depends on market penetration of DDGS across swine producers in the United States. If 100% market penetration of DDGS in swine feed is achieved, total losses to the swine industry from weight gain reduction alone from fumonisin is in the hundreds of millions of dollars.

In 2006, only 12% of swine operations used co-products in feed and included DDGS at an average of 10% (*72*). Therefore, the expected additional loss to the U.S. swine industry due to fumonisin in DDGS in 2006 is about $9 million through reduced weight gain ($2–18 million; hereafter, all values presented in parentheses represent lower−upper bounds). This assumes that the 10% of swine operations represents 10% of total animals, which may be an underestimate as the larger swine operations in Iowa are located near DDGS production sources and may utilize relatively more DDGS. There has been a recent proliferation of studies on the use of DDGS in swine diets (e.g., ref *73*), and it is likely that both market penetration and proportion of DDGS will increase in the immediate future. The USDA reports that in 2006, an additional 35% of swine operations were considering feeding co-products (*72*), suggesting that a market penetration near 50% could be reached in the near future. According to our model's estimates, if DDGS is included at 20%, this would amount to about a $74 million ($15–147 million) annual loss to the swine industry due to reduced weight gain from fumonisin in DDGS.

The economic losses estimated in **Table 4** represent a small fraction of the total value of the swine industry. However, these are the effects of just one mycotoxin on one adverse effect (reduced weight gain) in one species. Summed across

all mycotoxins, adverse effects, and species, the annual economic loss to livestock industries across the United States could be much higher if more widespread adoption of DDGS in animal diets occurs. Our model can be adapted to accommodate the impacts of more than one mycotoxin, as the parameters will have the same relationships regardless of the effects of each individual mycotoxin on different animal species.

Estimated Losses If Ethanol Producers Reject Maize with Highest Mycotoxin Levels. If surveillance systems are implemented to monitor for mycotoxin content at various points from farm to ethanol plants to animal feed, then an economic impact analysis is needed to assess which stakeholder groups will suffer economic losses from mycotoxins and the magnitude of those losses. Maize growers may lose through lower prices for maize that has excessively high mycotoxin concentrations. Ethanol facilities may lose through not being able to sell DDGS with excessively high mycotoxin levels, and/or they may need to pay higher prices for maize that is relatively clean. Livestock industries, aside from suffering economic losses due to potential animal health effects, may need to pay higher prices for both high-quality maize and high-quality DDGS for animal feed. Depending on where in the feed chain analytical tests for mycotoxins are done, any or all of these groups will also incur testing costs.

Suppose, for example, that ethanol plants were to reject the 5% of maize with the highest mycotoxin levels and that this maize were used for animal feed rather than for ethanol and DDGS production. It is difficult to estimate what the economic impact would be on corn growers, if any; maize sold for feed might command the same price as maize sold for ethanol, resulting in no net loss; there may be increased transportation costs for growers; or there may be a reduced price received for contaminated grain. The more relevant economic components are the analytical costs to the ethanol plants (which may or may not be offset by a price premium for high-quality DDGS) and the benefits to livestock and poultry from feed with lower mycotoxin levels.

In the example of swine weight gain reduction described above, eliminating the top 5% of mycotoxin-contaminated DDGS may *not* result in a large benefit in terms of improved weight gain, because of the shape of the dose–response curve for fumonisin B_1 and weight gain reduction: above 1 mg/kg fumonisin B_1, the marginal effect of increased dose is not as large (*19*). The improved weight gain may provide at most a benefit of $10 million to recoup the most severe losses described in **Table 4**. The real benefit from this type of intervention (removing the most highly contaminated grain at ethanol plants) would be reduction of more serious physical conditions, such as porcine pulmonary edema at higher fumonisin doses.

It is also important to consider the potential impact of a "shock event"—if mycotoxins in DDGS are shown to be so dangerously high that animal illnesses become a news-breaking event. Lawsuits, media frenzy, and subsequent policies may impose large costs on these stakeholder groups. Ultimately, the solutions lie in better mycotoxin control.

MANAGING MYCOTOXINS IN MAIZE AND SUBSEQUENT DDGS

Preventing mycotoxin accumulation in maize grain can be addressed through multiple tactics (reviewed in refs *74* and *75*), whether or not the grain is destined for ethanol production. Like most diseases of maize, the ear rot diseases that result in mycotoxin contamination can be managed through genetic resistance, cultural, biological, chemical, and physical control methods. Postharvest handling of grain intended for ethanol production offers additional challenges, but also opportunities to minimize the ultimate mycotoxin levels found in DDGS. An integrated, multifaceted strategy incorporating pre- and postharvest tactics will likely be the most successful approach.

Since the ascent of hybrid maize production, disease management in this crop has focused on genetic resistance, and there are opportunities to reduce the risk of mycotoxins in DDGS through genetic resistance to ear rot diseases caused by *Fusarium* and *Aspergillus* species. Each of these diseases (*Fusarium* ear rot, *Gibberella* ear rot, *Aspergillus* kernel rot) has been studied more or less intensively in relation to sources of resistance, genetic basis, and inheritance of resistance (*74–79*). Maize hybrids with partial resistance to all three diseases are available, but a greater emphasis on developing commercially acceptable resistant hybrids will be needed to consistently ensure acceptable mycotoxin levels in grain intended for ethanol production. Genes from organisms other than maize may be useful for resistance against ear rot diseases or for preventing or detoxifying mycotoxins, and researchers are actively pursuing effective genes that may be introduced into maize through genetic engineering (*75*). In fact, this strategy has already proved to be successful (although not commercially available) for fumonisins (*80*). Efforts to modify grain composition through genetic engineering for specific end uses must go hand-in-hand with attention to ear rot resistance.

There are other methods of genetically engineering maize to indirectly reduce mycotoxin levels. Insect injury predisposes corn to mycotoxin contamination, because insect herbivory creates kernel wounds that encourage fungal colonization, and insects themselves serve as vectors of fungal spores (*80, 81*). Thus, any method that reduces insect damage in maize also reduces the risk of fungal contamination and subsequent mycotoxin accumulation. The availability of transgenic insect-resistant maize (Bt maize) has therefore provided an opportunity to reduce the risk of mycotoxins in maize grain. Transgenic Bt maize contains a gene from the soil bacterium *Bacillus thuringiensis*, which encodes for formation of a crystal (Cry) protein that is toxic to common lepidopteran maize pests.

Reduced levels of several mycotoxins, including aflatoxins, deoxynivalenol, fumonisins, moniliformin, and zearalenone, have been reported in Bt maize (*82–91*). The most consistent and dramatic effects have been with fumonisins. Indeed, in a variety of field studies, Bt maize has been shown to have significantly lower levels of fumonisins compared to conventional maize, in some cases a 10-fold reduction (*82–84*). Effects on other mycotoxins have been lower in magnitude than those reported for fumonisins. The link between Bt maize events and aflatoxin reduction has been less consistent, with some studies showing a significant reduction and others showing no significant effect (*85, 87–89*). However, new events of Bt maize are being developed to resist these insects that predispose corn to aflatoxin contamination (*90, 91*).

With continually improving transgenic insect control, it is likely that Bt maize, if processed for ethanol, will yield co-products with significantly lower levels of mycotoxins than non-Bt maize co-products. The economic benefits of Bt maize to reduce mycotoxins in U.S. maize have already been shown to be substantial (*92, 93*).

Biocontrol methods specifically for aflatoxin reduction in maize have also shown field success. Multiple strains of atoxigenic *A. flavus* have been found that could inhibit aflatoxin production in vitro, but one in particular, AF36, also inhibits

aflatoxin production in the field, through competition with toxigenic strains (*94*). AF36 has been shown to have a defective polyketide synthase gene (*95*), which prevents aflatoxin biosynthesis. Inoculating maize with atoxigenic strains of *A. flavus* has been shown to reduce aflatoxin contamination (*96*).

Cultural practices designed to reduce mycotoxin contamination of crops have their roots in plant disease epidemiology. The general strategy is to alter the conditions under which the crop is grown so those conditions are less favorable for infection. Cultural control tactics include tillage practices, fertilization practices, crop rotation, plant population, planting date, and irrigation. Individual or combined effects of various cultural practices have been investigated for all three major mycotoxin-producing fungi in maize (*74, 75*). In general, these methods are partially successful, especially when multiple tactics are applied together. The challenge for crop producers is to balance practices that maximize yield with those that reduce mycotoxin risk. One particular concern is that, given the current demand for maize-based ethanol, growers may plant maize in successive seasons ("corn-on-corn" planting) rather than rotating with other crops, which may raise the risk of fungal diseases.

Postharvest mycotoxin development can occur prior to processing if grain is not handled and stored properly. Prevention of mold growth and mycotoxin development depends on maintaining kernel low moisture and temperature, minimizing kernel damage, sanitation, insect control in storage, and frequent monitoring (*97*). The growing bioeconomy may present new challenges in this regard as the supply of maize grain outstrips existing storage capacity. Mounds of grain stored outdoors are vulnerable to degradation by molds and contamination by their mycotoxins. A sustained ethanol industry that utilizes maize grain will require the construction of additional storage facilities.

The use of grain for ethanol production may offer some unique postharvest mycotoxin management strategies involving detoxification, grain cleaning, and/or fractionation of kernels prior to processing. Several approaches to detoxification of grain by physical, chemical, and biological mechanisms have been relatively successful. Use of some methods can be constrained by undesirable or unknown impacts on fermentation or food/feed safety (*98*). The kernel component targeted for fermentation includes only the endosperm starch. In undamaged kernels, mycotoxin-producing fungi and their mycotoxins are found primarily in the pericarp and germ portions of the kernel (*99, 100*). Therefore, DDGS consists of material derived from the lower-mycotoxin endosperm residue combined with the higher-mycotoxin pericarp and germ. Separation of these components (kernel fractionation) prior to fermentation could be advantageous for increasing the value of co-products. Through a dry-milling process, the pericarp and germ can be removed for use as separate high-fiber and high-oil co-products, removing these materials before fermentation, so that the postfermentation DDGS would consist of unfermented endosperm material, typically low in mycotoxins and high in value as a livestock feed component. Bran and germ fractions, if high in mycotoxins, can then be used as biofuels. This approach has been demonstrated for aflatoxin-contaminated maize in Zimbabwe (*101*); dehulling maize kernels reduced aflatoxin concentrations by an average of 92%. Similarly, 40–100% of DON and zearalenone was removed from barley and maize grains when they were dehulled (*102*). Smaller particles (broken kernels) in maize lots, which can be removed by sieving, are known to be higher in aflatoxins (*103*), DON, zearalenone (*104*), and fumonisins (*15*). Hence, cleaning grain prior to ethanol processing could be an effective way to reduce the risk of mycotoxins in DDGS.

Monitoring mycotoxins in grain and grain products is an important part of the overall strategy for preventing exposure to mycotoxins in food and feed (*105*). Monitoring mycotoxins and diverting highly contaminated raw products to lower-risk uses can be a direct way of reducing the overall risk of detrimental health effects. In the ethanol co-product system, monitoring can be implemented by the grain producer, the ethanol producer, the co-product distributor(s), or co-product end-user. The distribution of costs for monitoring and finding alternative uses may be complicated, as was already discussed. Although it is likely that reductions in co-product mycotoxin levels are being achieved and could be enhanced, the costs of diverting large amounts of grain away from the increasing demand for ethanol could be prohibitive. As already discussed, the dose–response relationship for swine exposure to fumonisins dictates that a significant proportion of the most highly contaminated grain must be diverted to achieve a meaningful improvement in animal health.

Another approach that may drastically reduce the mycotoxin problem in ethanol coproducts is to produce ethanol from sources other than maize kernels. One often-cited solution is ethanol production from cellulosic sources such as switchgrass, trees, or alfalfa. The problem has been the difficulty of breaking down cellulose for ethanol processing. In August 2006, the U.S. Department of Energy (DOE) announced the creation of two centers focused on biofuels that would include studies of microbes that digest cellulose, the development of transgenic plants that would be easier to break down, and the design of new fermentation processes (*106*). Although cellulosic courses are not always free of mycotoxins themselves, levels are considerably lower (*107*).

SUMMARY

As ethanol production increases over the next decade, there will be a substantial increase in the amount of ethanol co-products used in livestock and poultry feed. This presents a potential animal health risk, because mycotoxins in the original grain are concentrated up to three times in the co-products, as verified by studies across multiple mycotoxins. Mycotoxins cause a variety of adverse health effects in livestock and poultry. In swine, for example, the mycotoxin fumonisin causes reduced weight gain even at relatively low doses and can cause pulmonary edema and other more severe clinical signs at higher doses.

We developed a model to estimate the economic impact to livestock and poultry industries from including dried distillers' grain and solubles (DDGS) in animal feed. The impact depends on multiple factors, including the mycotoxin concentration in the original grain, the proportion of DDGS in the diet, animal health effects, and the market value of animals. We applied this model to one specific case study: the impact of fumonisin in reducing weight gain in swine. At current levels of DDGS usage in swine feed across the United States, the annual loss to the swine industry from this reduced weight gain is about $9 million, based on a 20% DDGS incorporation in the feed. If there were full market penetration of DDGS in swine operations (in which DDGS would comprise 20% of the swine diet on average), the estimated loss due to reduced weight gain could reach hundreds of millions of dollars annually. In reality, to understand the complete impact of DDGS in swine diets, one would have to sum across all possible health effects and all relevant mycotoxins.

This model has several limitations. First, it does not account for mycotoxin mitigation strategies that occur between the production of the DDGS and the sale to livestock and poultry

industries. It also does not account for the fact that ethanol facilities may have surveillance programs in place to reject grains that contain excessively high mycotoxin levels. For this reason, we also considered what would happen if grains with the top 5% of mycotoxin levels were rejected. Swine weight gain reduction might not be substantially mitigated, but more severe health effects probably would be. It is possible to apply sensitivity analysis to the parameters of this model to estimate impacts under different conditions of mycotoxin control and animal effects.

There are many pre- and postharvest strategies that can manage mycotoxin levels in maize for improved co-product quality. These include cultural, biological, genetic, chemical, and physical means: good agronomic practices, conventional or transgenic plant breeding for improved host resistance, biocontrol methods (to reduce aflatoxin), improved storage conditions, kernel cleaning and fractionation, and monitoring methods. All of these methods have the potential to reduce final mycotoxin concentrations in DDGS, resulting in improved animal health. These methods will become increasingly important as DDGS and other ethanol co-products play an ever-more important role in animal feed in the near future.

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