Mycotoxin Detection and Solutions

Donald W. Giesting, Ph.D.

Provimi North America/Cargill Animal Nutrition
15407 McGinty Road West, MS 14, Wayzata, MN 55391, USA
Don_giesting@cargill.com

1. Introduction

Mycotoxins are a threat to the health, performance, and economic viability of all commercial animal businesses. More than 250 different mold toxins have been identified, but a much lower number occur regularly and have meaningful physiological impact on animals. For swine producers in Canada, this list can commonly be truncated further to focus on the predominant culprits: deoxynivalenol (DON) and zearalenone (ZEA). These toxins can be produced by multiple species of the *Fusarium* genera. Prominent among fusarial species for production of both DON and ZEA is *Fusarium graminearum*, sometimes referred to as *Gibberella zeae.* There are multiple compounds related to DON that may be partially responsible for the negative impacts on animal production commonly associated with DON contamination.

Mycotoxins have multiple metabolic impacts, including damage to internal organs, disruption of reproductive functions, suppression of immune response, imbalance of antioxidant systems, and predisposition of animals to secondary disease impacts when bacterial or viral stressors are present. The resultant measurable effects are reductions in animal performance, decreased reproductive efficiency, lower pig viability and survival rates, and higher morbidity and mortality throughout the growing period. Of course, these effects can strongly impact the economic viability of swine enterprises.

2. Risk Assessment

When considering how to estimate and control the risk of mycotoxins affecting swine performance and health, several aspects need to be considered. First, assess the prevalence of the molds producing the toxins and conditions favoring mycotoxin production by the molds; second, determine the effects of the mycotoxins and levels triggering damage, sometimes referred to as action levels; and third, establish meaningful estimates of mycotoxin levels in grains, grain byproducts, or final feed being consumed by animals.

2.1 Mold Prevalence

It is generally accepted that in the northern United States and Canada, the molds producing DON and ZEA are ubiquitous. Levels may be higher in some years, in minimum tillage cropping systems, during some phases of plant growth, and during weather periods that favor mold growth. However, the general observation is that *Fusarium* species are always in the fields. The molds generally produce mycotoxins as a defense mechanism in response to stress. Thus, while mold at a meaningful level is a necessary prerequisite for mycotoxin production, the level of mold is not a good predictor of mycotoxin prevalence. For example, *Fusarium* molds commonly produce toxins when their environment becomes wetter and cooler than their optimal mold growth conditions. By contrast, *Aspergillus* species produce aflatoxins when conditions become dry and hot. Managing “field” toxins, which are produced before plants are harvested, is very difficult. Plant pests and diseases may exacerbate toxin production, thus, wheat with bunt or rust is more prone to *Fusarium* toxins, and corn affected by rootworms or earworms may be more vulnerable to mycotoxin formation, as well.

2.2 Effects of Deoxynivalenol and Zearalenone

2.2.1 Deoxynivalenol and related Tricothecenes

There is a wide array of *Fusarium* toxins, and the tricothecenes group is generally the most problematic. Tricothecenes of primary consequence are part of two main families. Type A tricothecenes include T-2 and HT-2 toxins, and Type B include DON, nivalenol, and several variants with different acetyl and hydroxyl groups on particular carbons in their structure. Type B tricothecenes induce immunosuppression, challenge antioxidant systems in the body, and inhibit protein synthesis. Deoxynivalenol, sometimes called vomitoxin, and nivalenol appear to be the most common and problematic tricothecenes affecting swine in the U.S. and Canada. Most *Fusarium* toxins are primarily “field” toxins that do not generally increase in storage, unless moisture content is quite high.

The reported effects of DON are often observed when other, related toxins are present because DON is rarely found alone in feedstuffs. This contamination causes obvious reductions of feed intake and resultant poorer gain or lactation performance. Impacts on immunity, antioxidant status, and serum protein levels may have less obvious, but more insidious effects on animals, especially when exposure to DON is chronic. Increases in secondary disease risk, unthrifty pigs, sow reproductive failure, or culling for other reasons have been observed. Levels in excess of 1 ppm DON have been noted to produce modest intake reductions in naïve swine, particularly young pigs. Effects increase with graduated increases in DON contamination to a point that consumption in excess of 4-5 ppm will reduce intake 25 to 50% in newly exposed animals.

2.2.1 Zearalenone

Zearalenone is an estrogenic compound produced by multiple species of *Fusarium*. It is highly problematic when fed to developing gilts and reproducing sows. In female growing animals, ZEA can cause premature mammary and genital development. Levels of ZEA greater than 0.5 ppm are generally considered to produce limited risk of reproductive changes, and levels higher than 1 ppm are potentially problematic in most reproducing swine.

2.3 Monitoring Mycotoxin Levels

As previously mentioned, determining the changes in production and animal health to specific levels of mycotoxins can be fraught with challenges because animal performance is also concurrently affected by other factors, including environmental, social, and disease stressors. Animals in low stress conditions often appear to have much higher tolerance for mycotoxins than those in commercial production situations. Further complications arise from the challenge of determining the approximate toxin load being consumed by animals. Because molds grow from spores that are unevenly dispersed and subject to different micro-environments, especially moisture and temperature, it is common to have highly variable concentrations of toxins from different sampling locations within a field of grain, in a lot of grain from storage, and in feed after further processing. Additionally, most research indicates that naturally-occurring toxin contamination produces more severe effects than addition of purified toxin to test diets. Some ascribe this phenomenon to “hidden” forms of toxins that are not measured by most quick-test methods, or related compounds that may not be noted in analyses. Further complicating assessment is the reality that while analytical methods have improved, and relatively safe, simple, inexpensive testing methods have become widely available, the greatest single challenge to mycotoxin assessment is sampling. Due to the disparate levels occurring at different points in handling and storage systems, the sampling technique applied is critical to representative estimates of mycotoxin levels. In general, taking at least 50 large (more than 500 gram) samples from within a storage or grain handling container is recommended. Sampling flowing grain is often easiest and best to obtain representative samples. In turn, blending these multiple samples and sub-sampling for a final sample to be analyzed is recommended to best represent the lot of grain. In turn, frequent sampling at time of storage and pooling results from samples within a geographical region where grain and grain byproducts are sourced are powerful ways to improve accuracy of the assessment of the mycotoxin risk facing a swine enterprise. Surveys can be very useful, but require meaningful sample numbers and good sampling near to grain or feed sources to be predictive.

3. Reducing the Risks and Impact of Mycotoxins

Given the prevalence and potential impact of mycotoxins, managing risk to reduce impact is critical to success, especially during seasons when mycotoxins are plentiful. Whether you are purchasing complete feeds or inputs to generate final rations, the principles are the same:

- Avoid contaminated ingredients as much as possible, through appropriate, risk-based monitoring.
- Invest in good handling, storage, and mold inhibition programs to prevent deterioration or an increase in contamination within ingredients or final diets.
- Utilize available technological ingredients to limit impact of unavoidable toxin effects.

While neither Canada (CFIA) nor the U.S. regulatory agencies currently have established ingredient categories for the reduction of mycotoxin effects, some ingredients which have other benefits may also provide some support for animal health and performance in the face of reduced quality grain affected by molds and mycotoxins. These ingredients may include compounds which naturally bind to mycotoxins and reduce their rate of uptake from the gastrointestinal (GI) tract, produce changes in mycotoxin structure to reduce toxicity, or support animals in some other way to reduce deleterious effects of absorbed toxins. Products containing a combination of two or more of these components are common.

3.1 Reducing Deoxynivalenol Effects

While many toxins can be bound by clays, yeast derivatives, chitosans, or other molecular sieves, DON and related compounds are very difficult to bind and hold throughout the GI tract. Various companies have pursued technologies to modify DON to less toxic forms. Microbial or enzymatic modification has been widely touted, but much neutral, third-party testing leaves doubts of *in vivo* efficacy of such products. This lack of response may relate to the difficulty connecting limited enzyme or microbial concentrations to mycotoxins immersed in a mixed matrix of feed and digestive secretions in the GI tract. More encouraging have been results of sulfur-containing preservative compounds that modify DON in the GI tract and have been shown to strongly reduce the impact of DON (Table 1).

Table 1. Comparison of various commercial products in high DON, wheat-based pig diets.

	Control	DON-Control	DON + Yst Drvt	DON + MBld	DON + ALU	DON + PrvBld	SEMy
0-14 days							
14-day BW (kg)	12.14	9.87*	9.90*	10.64*	9.98*	11.63	0.79
ADG (g d^{-1})	373	220*	224*	263*	236*	352	29
ADFI (g d^{-1})	497	390*	417*	440	390*	444	41
G:F	0.76	0.58*	0.53*	0.61*	0.62*	0.79	0.06

Control diet (<0.50 ppm DON), DON-contaminated diet (4.61 ppm DON), DON + Yst Drvt diet (4.71 ppm kg^{-1} DON + 1g kg^{-1} yeast derivative product), DON + MBld diet (4.21 mg kg^{-1} + DON + 1.5 g kg^{-1} Multi-form blend), DON + ALU diet (4.68 mg kg^{-1} DON + 2.5 g kg^{-1} aluminosilicate) and DON + PrvBld diet (3.03 mg kg^{-1} + 2.5 g kg^{-1} preservative blend).
*Indicates that LSmeans are significantly different from the control diet, $P < 0.05$.
**Indicates that a P value between 0.05 to 0.10 may be considered as a trend toward significance.
Thanh, et al, Canadian J Anim Sci (2015) 95:197-209.

While this method appears to produce superior performance enhancement to binders or microbial or enzymatic modifiers of toxins, in most studies performance remains below unchallenged controls. Recent evidence from Provimi shows the effects of sulfur-containing preservatives can be augmented by complementary addition of other compounds to address the depressing effects of DON on antioxidant status and immunity.

Table 2. Effects of Preservative blend, immune support and antioxidant support on performance and economic response of young pigs fed low or high DON diets.

	No Immune Support High DON			*0.1 kg/MT Immune Support High DON*								
0-18 days	**0**	**1X Antiox**	**2X Antiox**	**0**	**1X Antiox**	**2X Antiox**	**Clean Diet Control**	**SEM**	**None vs Antiox**	**1X v 2X Antiox**	**Imm sprt**	**Clean vs treated**
Initial wt, kg	12.47	12.46	12.46	12.45	12.51	12.45	12.49	0.42	ns	ns	ns	ns
Final wt, kg	20.29	20.57	20.93	20.54	20.96	21.23	22.09	0.31	***	*	**	***
ADG, kg/d	0.436	0.451	0.471	0.450	0.470	0.489	0.523	0.014	***	**	**	***
ADFI, kg/d	0.607	0.616	0.629	0.595	0.629	0.645	0.693	0.017	***	*	ns	***
Gain to Feed	0.718	0.734	0.749	0.756	0.748	0.757	0.763	0.009	ns	ns	***	***
Cost/kg of gain, USD/kg	0.489	0.479	0.470	0.466	0.473	0.467	0.457	0.006	ns	ns	**	***
Return over feed cost, USD/pig	13.30	13.98	14.64	14.01	14.62	15.19	16.75	0.52	***	**	***	***

While the specific sulfur-containing preservatives shown to be effective against DON are permitted in feed in the U.S., they are not currently approved for use in swine diets in Canada. Efforts are underway to secure clearance by CFIA. Versions of the compounds shown to be complimentary to sulfur-containing preservatives are available now in the U.S. and Canada. Thus, at least a partial solution is available in both countries, but will be strongly enhanced once the sulfur containing preservative product is permitted in Canada.

3.2 Reducing Zearalenone Effects

Zearalenone structures can be bound by some clay-type products properly treated to optimize binding of more polar toxins. Additionally, other compounds have shown some effect in binding ZEA.

While *in vivo* measurements of binder effects in the presence of ZEA do not always verify *in vitro* binding results, controlled and practical field studies indicate reductions in reproductive effects in pigs challenged with ZEA.

Table 3. *In vitro* percent binding efficiency* comparison.

	Adsorbent inclusion (kg/MT)	4000 ppb Aflatoxin B1	5000 ppb Zearalenone	3000 ppm Fumonisin B1	2500 ppb Ochratoxin A
Clay 1, not modified	5	98	34	98	53
Specialized carbon source	2	0	41	51	58
Modified yeast cell walls	1	39	26	3	67
Clay 2, partially modified	3	76	95	95	93
Clay 3, modified	1	61	95	33	89
Clay 4, modified	1	92	94	93	92

Unpublished, Internal Cargill Data
*Efficiency = binding at low pH, resuspend at higher pH, subtract loss during desorption to calculate efficiency.

Table 4. Reproductive tract length, cervix diameter, and vulva volume in young gilts fed low or high DON diets.

Treatments	Length (cm)	Cervix diameter (cm)	Volume of vulva
PC	31.18 A	0.825 A	3,067 A
DON Challenged	40.25 B	1.925 B	10,077 B
DON + Modified clay	34.23 AB	1.275 A	6,678 AB
DON + Combination product	36.48 AB	1.325 AB	9,090 B

AB Values without common superscript differ (p<.05).

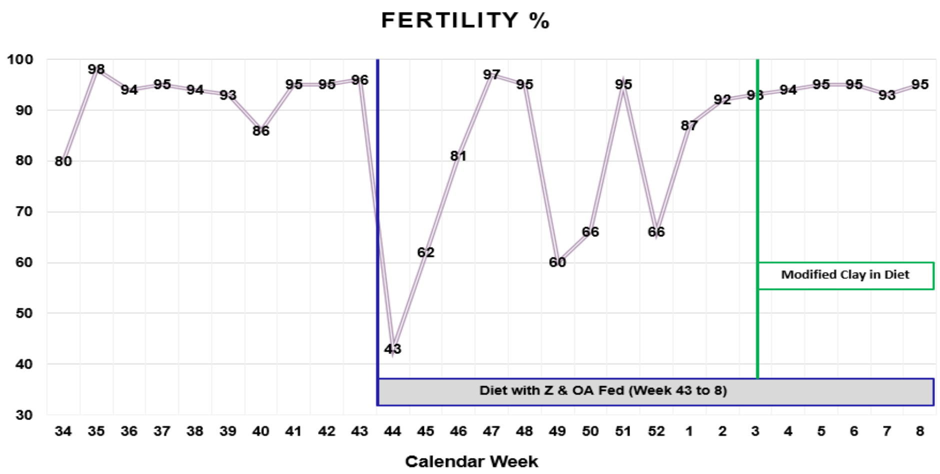

Figure 1. Fertility response of sows to modified clay with ZEA and OTA in diet.

The modified clay shown to most effectively bind ZEA is permitted in Canada and the U.S.

4. Conclusions

Avoiding the pitfalls of molds and mycotoxin contamination requires a systematic approach to prevention. Reliable feed suppliers are implementing, monitoring, and managing approaches similar to those described in this paper. Producers formulating and manufacturing diets must understand and develop an operative and cost-effective system to avoid mold and mycotoxin problems.

While no animal feed can be guaranteed to be free of toxins, three key principles can be applied to reduce the risk and impact of molds and mycotoxins:

1. Monitor raw materials to understand contamination risk.
2. Manage materials appropriately to reduce risk of further toxin formation and minimize feeding of higher risk ingredients or feeds to more vulnerable animals (young pigs and reproducing sows).
3. Utilize appropriate ingredients to ameliorate the effects of contamination when risk is unavoidable.

References

Thanh, V.T.L., M. Lessard, Y. Chorti, F. Guay. (2015) The efficacy of anti-mycotoxin feed additives in preventing the adverse effects of wheat naturally contaminated with Fusarium mycotoxins on performance, intestinal barrier function and nutrient digestibility and retention in weanling pigs. Can. J. Anim. Sci. 95:197-209.