#### MYCOTOXINS: LESSONS LEARNED FROM THE 2009 MIDWEST CROP YEAR

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#### **INTRODUCTION**

The combination of delayed planting, cool summer, and a rainy fall weather pattern that delayed harvest created a harvest situation predisposing the 2009 crops to mold and mycotoxins. In many areas, October rainfall and cooler than average weather badly hampered the Midwest corn harvest in 2009. October is the most important month for corn harvest, with average production records from 2004 - 2008showing 70 % of the corn usually harvested by the end of the month. However, in 2009 only 25 % of the corn was harvested by the end of October and as a result standing corn was at risk of mold and mycotoxin formation. Corn normally dries 0.25 -0.5 % units/d in October to early November in the Midwest, with November providing much less drying opportunity. In 2009, by mid-November as much as 45 % of the corn was still not harvested nationally, requiring more energy driven drying to take much of the corn from moistures, often nearing 30 %, to the required 15 % or lower. Drying corn this wet coming out of the field required aggressive drying practices potentially resulting in a higher percentage of damaged kernels.

While the corn crop harvested in 2009 may have looked normal or clean to the naked eye, the potential and predisposing factors for mold and mycotoxins certainly existed. Thankfully, the feed testing expertise and industry infrastructure has advanced to a point where nutritionists, veterinarians, extension, and laboratories were on high alert as to the need to characterize and monitor the 2009 crops for mycotoxins. While the awareness was high regarding the potential quality issues with the 2009 corn crop, the journey was bumpy and in some cases expensive trying to manage the feeding for the following year. There's an old saying along the lines of what doesn't kill you will make you stronger. Certainly this statement might apply to the lessons learned from feeding the 2009 corn crop throughout the country and in the Midwest.

The purpose of this paper is to share key lessons learned during late 2009 and most of 2010 from

having to manage and feed the compromised 2009 corn crop to dairy cows. Admittingly, many of these lessons are not deeply backed by controlled data, but hopefully can still serve those faced with a similar challenge in the future. Our observations and recommendations are from a more practical and analytical perspective, rather than animal research, operating both as an independent nutrition consultant<sup>1</sup> and as the manager<sup>2</sup> of a large Midwest-based forage and feed testing laboratory.

#### MOLD AND MYCOTOXIN BACKGROUND

There are many excellent papers published on the scientific background of mold and mycotoxins, which can be referred to outside this paper. One resource center to consider would be <u>www.dairylandlabs.com</u>. Moldy and musty feed won't always contain mold poisons or *mycotoxins*, but the presence of mold itself may adversely affect production and health. For example, more problems with mycotic abortions and respiratory disorders may result when moldy feed is used. This may be due to a high content of mold ingested and/or mold spores in the air. Mold spore count of moldy feed can be an indication of the extent of mold contamination and the relative risks of feeding or using the moldy feed for bedding.

Mycotoxins are harmful toxic compounds produced by molds or fungi. They are found in soil and can grow on both grains and forages. Molds and mycotoxin production can occur in the field during the growing season, during the harvest season as the crop is drying in the field, and post-harvest while in storage or during feed-out. Myco means fungus and toxin meaning poison. Mycotoxins are poisons generated from the secondary metabolic processes and growth of mold. Not all molds produce mycotoxins (consider some types of tasty cheese are created from selected molds). The amount and type of mycotoxin varies with environmental conditions and specific growing conditions that involves temperature and rainfall patterns; damaging weather conditions, such as hail and strong winds; and insect

infestation challenge levels. These factors have proven hard to model and accurately predict the level of mycotoxin risk.

Ruminants are generally most resistant to mold and mycotoxins due to the rumen microorganisms likely being capable of degrading and metabolizing some of the molds and fungi prior to entering the small intestine and bloodstream. However, there are broad data and research documenting detrimental effects on ruminants such as reduced feed intake, inconsistent gut health, reduced nutrient utilization, suppressed immunity, altered rumen fermentation, reduced fiber digestibility, reduced reproduction, overall reduced disease resistance, and higher culling rates.

There are literally hundreds of mycotoxins that have been identified, yet only a few are regularly tested and quantified in the lab. Again, the purpose of this paper is not to detail the different types of mycotoxins, with information readily available in the literature. The mycotoxins of greatest concern in the 2009 wet harvest conditions were vomitoxin (**DON**), zearalenone, and T-2; all of which are from the Fusarium group of molds. While aflatoxin has been the most researched of the mycotoxins, it is typically most problematic in hot, humid regions and more limited to crops grown in the southern states of the US.

Fusarium mycotoxins are broadly classified into trichothecene and non-trichothecene groups. Trichothecenes include DON and T-2 mycotoxins. The most common Fusarium is DON and is believed to affect dairy cows by causing digestive upsets and reduced nutrient absorption including possibly bloody gut related issues (Smith, 2010 and personal communication). It has been suggested that DON is related to reduced feed intake causing immunosuppression and greater susceptibility to mastitis (Smith, 2010).

A significant problem when researching single mycotoxins is they almost always are found in feed as multiple mycotoxins. Having this naturally occurring combination of mycotoxins makes them capable of exerting an additive effect (Smith, 2010). One might think of this like the mixing and consumption of alcohol in combination with prescribed medications with significant consequences. This makes quantifying a *toxic* threshold for a single mycotoxin difficult at best, and at times impossible. What may appear to be a moderate level of a single mycotoxin, as determined by laboratory analysis, may in fact be exerting acute and clinical symptoms on the cows due to the aggregate effect of multiple mycotoxins (many of which are not measureable or tested for in the laboratory).

The effects of mycotoxins can be acute due to a toxic dose short-term from a heavily contaminated feed or feeds with immediate symptoms such as reduced milk production, lack of normal reproductive cyclicity, elevated somatic cell count (SCC), and digestive upsets with inconsistent manure patterns. However, the effects of mycotoxins are often an accumulative problem (Adams et al.) over time with the animal *toxicity* being more of an expression of chronic metabolic fatigue and response to a chronic moderate level of mycotoxins that may or may not even be measureable on a consistent basis. In fact, the author's experience in the field has been more with the chronic effects more than acute and sudden challenges. It appears that it often can takes weeks of mycotoxin intake to cause marked changes in performance.

#### **MIDWEST 2009 CROP CHALLENGE**

When sampling for mycotoxins it's difficult to know how to take representative samples; since mycotoxins are present in such small quantities and can be in isolated spots in the bunker, bag, silo, or bin. In the case of wet by-products, such as wet corn gluten feed and wet distillers grains, the turnover of the inventory is so great that the feed is almost always fed up and gone by the time the laboratory results are available. In the case of corn silage, due to the mass of forage in a pile or bunker it is difficult to gather a representative sample, where literally a 1 – 2 lb sample may represent over 100,000 lb or more of wet corn silage being fed daily. With the total mixed ration (**TMR**), again the relatively small sample size compared to the mass of TMR being fed daily is a challenge. Additionally, the dilution factor of each ingredient being only a portion of the ration reduces the level of mycotoxin at times below a threshold level of detection on a given day of feeding. In other words, a single problematic feed ingredient being fed at a level less than 10 - 20 % of the ration dry matter (DM) may deliver a measureable or concerning level of mycotoxin one day, yet not the next.

With commercial laboratories now offering database summaries of analytical data, it's most helpful to review this type of data as background information to help address feeding and nutrition related questions with actual laboratory data over a large population of samples. However, when looking at the prevalence of mycotoxins from laboratory



Table 1. Midwest 2009 mycotoxin results – corn grain samples submitted Oct 1 – Dec 3, 2009.



databases, one must be reminded that the data likely does not represent a normal random population. This is simply based on the fact the samples were probably selected based on other known information, such as visual mold. This does not suggest the database information isn't valuable, quite the opposite. Rather, it just must be remembered the data does not appropriately allow one to draw conclusions about the prevalence of different mycotoxins for the entire crop harvest in a given area because you do not have a random population of samples. With this said, Tables 1, 2, and 3 from Dairyland Laboratories (personal communication, Dave Taysom) give an excellent illustration of the magnitude of the problem faced in the Midwest in 2009.

#### **LESSONS LEARNED IN 2009 – 2010**

Lesson 1- Take advantage and learn from resources available, multi-media has opened a whole world of information at our finger tips. To the credit of the university extension service dairy professionals in the US, and the expertise within commercial organizations serving the dairy industry, there was a tremendous amount of excellent resources available immediately in the fall of 2009 to help address the mycotoxin challenge. For those well-traveled, this is a point which is often easily taken for granted; the US dairy producer and industry has a wealth of talented professionals, resources, and services to serve.

# Lesson 2 – High prevalence of mycotoxins in a region doesn't mean all challenges with cows or dairies all involve mycotoxins.

During the 2009/2010 time period some dairies experienced more than normal gut health issues, and in some cases more challenges with dead cows and higher than normal culling, that were in fact related to the documented levels of mycotoxins. This said, a high percentage of Midwest dairies did not face mycotoxin challenges through a combination of good fortune with the weather, or the ability to eliminate the feeds of concern. In some cases, even with

Table 2. Midwest 2009 mycotoxin results – corn silage samples submitted Oct 1 – Dec 3, 2009.



Table 3. Midwest 2009 mold results – summary 1743 samples submitted Oct 1 – Dec 3, 2009.

MOL	D IDENTIFI	CATION	Dairylaud Laboratories, Iuc.	
AIL II	Forag	ge: Shell Corn		1
	Dairyland La	bs Oct-Dec 3		
	Total # of Samples tested: 1275			
		Number of		
	Molds	Samples	% of Total Samples	
	Penicillium	140	10.98%	
	Aspergillus	110	8.63%	
	Mucor	199	15.61%	
	Rhizopus	86	6.75%	
	Fusarium	403	31.61%	
	Cladosporium	267	20.94%	
	Other	70	5.49%	
	Forage: <u>Corn Silage</u> Daryland Labs Oct-Dec 3			
	Total # of Samples tested: 468			
		Number of		
	Molds	Samples	% of Total Samples	
	Penicillium	61	13.03%	
	Aspergillus	58	12.39%	
	Mucor	131	27.99%	
	Rhizopus	26	5.56%	
	Fusarium	117	25.00%	
	Cladosporium	61	13.03%	
	Other	14	2.99%	Oct 1 Dec 2 2000

measurable levels of mycotoxin the cows did not seem to experience any negative outcomes associated with mycotoxins. The lack of a measurable mycotoxin impact may have been due to lower levels of mycotoxins or, more likely, mycotoxins that were present were less *toxic*. The duration of the exposure is always a consideration as well. In the author's experience, the dairy industry has often been swayed to using mycotoxins as an expensive *scapegoat* cause for lagging performance or health issues for what may in fact have been due to other underlying management bottlenecks or nutrition related problems. All troubleshooting on a dairy of any problem or challenge should always involve a broad based diagnostic approach and solutions.

#### Lesson 3 – You will need more than controlled dairy research to determine how much of a mycotoxin *iceberg* problem is below water.

Controlled research data is only one piece of information to help manage and navigate a mycotoxin challenge. The author's experience has been that using only published recommended tolerance threshold levels of mycotoxins, based on the scientific literature, may not adequately address the cow problems at hand. There are excellent controlled dairy research studies done on mycotoxins. However, much of this research has been done with aflatoxin versus fusarium mycotoxins, or where pure isolated single mycotoxins were tested rather than a more likely commercial dairy situation of having a multiple mycotoxin-based challenge. As mentioned, a mixture of mycotoxins likely has a much different impact than might be predicted from single source mycotoxin-based study. Another factor at play may be that controlled research is often conducted with relatively small populations of cows where environmental stress factors may be different than in larger groups of cows on a commercial dairy.

Sound science and valid controlled research data is always the foundation from which to start problem solving. In addition, we leveraged using well thought out early aggressive sampling and testing of ingredients, appropriate lab data interpretation, tight cow and herd monitoring, elimination of problematic ingredients, reduced feeding rates of suspect ingredients, and appropriate use of feed-based binders and additives. Each proved valuable in situations where recommended research-based mycotoxin tolerance thresholds were not exceeded, yet issues were faced with the cows that were most likely mycotoxin-based.

# Lesson 4 – Understand the detection limits of a given laboratory and the methodology of testing for a specific mycotoxin, knowing there are variations between laboratories and testing methodologies.

There commonly are two procedures used for testing mycotoxins, namely ELISA (enzyme-linked immunosorbent assay) and chromatography. Hence, there are inherent differences in the test results depending on the test procedure used and the type of feed being tested. Within chromatography testing there are HPLC, GC, and TLC methodologies being used at different labs. The detection limits for mycotoxins differ based on the type of mycotoxin being tested and the laboratory methodology being utilized. This is important to nutritionists and other involved professionals advising dairies on mycotoxin management strategies to understand what are the detection limits from the given lab. An example of this might be the high number of T-2 false positives reported in TMR samples because of the methodology used by some labs. In other words, T-2 was a feed sample concern, yet maybe wasn't a valid animal concern due to misinterpretation of the lab data.

#### Lesson 5 – Sampling of feeds requires more preplanning before taking samples than often occurs to minimize sample result misinterpretation.

Because feed variation can never be eliminated, unnecessary variation must be controlled (Taysom. 2008). Statisticians employ the term *error* to explain variation, however the word has the connotation that a mistake was made by someone. Really, the only error is not understanding the principles of variation. Numerous studies have documented that the analytical variation of feeds by a certified laboratory is generally quite small when compared to the sampling and actual feed variation that occurs at the dairy. Feeds inherently vary in chemical and biological composition due to genetics and environmental effects, while laboratories may use different analytical procedures to measure the same feed chemical entity. Variation is a given...how we choose to manage it when sampling and interpreting results on mycotoxins is the key. Detailed mycotoxin sampling guidelines are published (Adams et al.).

The approach is often to sample a feed, have it analyzed, and then formulate a diet based on that information. When a newer analysis is obtained, the

previous data is often eliminated and a new diet is formulated based on the new lab results. The inherent assumption of this is that the new data better represents the feed than did the older data (Weiss, 2007). This may or may not be true. When new feed sample data is obtained, the key question becomes "Is there good reason why the composition changed?" Possible answers might be a different supplier or plant, a different crop, a different storage structure, or "I don't know". If there isn't good cause for change, the reported lab difference may be caused by load-toload random variation, by within-load variation, or both (Weiss, 2007). In this case, the new data may be no more useful than the old lab numbers, while the mean of the two sample figures has the highest probability of being correct, assuming there wasn't a known change in the feed.

The principles of sampling and understanding basic probability and random populations was put to good use in 2009 when testing multiple lots of both corn gluten feed and wet distillers grains. Virtually all dairies the author consults with feed one or both of these ingredients. Rather than focusing on any one given load or a single dairy feeding either of these ingredients, we tested multiple loads instead, identified by plant, across all dairies over a relatively short period of time, and then using the mean results and standard deviation across all loads were able to determine that these corn-based byproducts were in fact a considerable source of concern for fusarium mycotoxins. This was some of the first data of this type known and reported in the Midwest in December 2009; well before the byproduct industry had addressed the concern. Based on this sampling data dairies reduced, or completely removed, the corn gluten feed and wet distillers grains. We have since gone back to feeding these ingredients at high inclusion rates with the 2010 crop year.

Other sampling guidelines recommended include:

- 1) focus on basal ingredients rather than the finished TMR to isolate where there may be a specific problem with one ingredient,
- use a silage defacer when available to get a more homogenous sample from the entire silage face being fed,
- take at least 10-12 same-sized subsamples representing the entire mass of feed being fed,
- 4) test multiple delivered lots if sampling a byproduct,
- 5) keep the sample cool at all times before reaching the laboratory if doing mold counts, and

6) take samples only of feed actually being fed in the ration versus top layer moldy feed or sidewall feed that is being discarded prior to feeding.

# Lesson 6 – Eliminate suspect ingredients rather than managing to a recommended tolerance threshold.

It's a challenge to know how to interpret a mycotoxin tolerance threshold recommendation given the diversity of animal responses in both controlled research and commercial herds. The inherent sampling variation that occurs, laboratory methodology differences, and data interpretation challenges all make tolerance recommendations tough. Hence, we found it much more effective to remove from the ration any suspect or welldocumented problematic ingredients rather than trying to manage to a recommended tolerance threshold figure.

#### Lesson 7 – Fumonisin mycotoxin was fairly commonly found in 2009 corn, yet the impact is not very well understood in ruminants.

Fumonisin mycotoxins are a dreaded toxin to horse owners, yet typically have not been associated or regularly identified as an issue with ruminants and dairy cows. Fumonisin was fairly common in sample results from Dairyland Laboratories (Taysom, personal communication), and may warrant some further investigation on how this mycotoxin may influence cow health or performance in the presence of other mycotoxins.

#### Lesson 8 – Expanded mycotoxin analysis beyond the more common fusarium mycotoxin test results (DON, T-2, zearalenone) did not appear to enhance troubleshooting.

It's well known there are literally hundreds of different mycotoxin compounds, each having a different chemical configuration. Routinely, only about 4 - 5 of the more common mycotoxins are tested for including: aflatoxin, DON, zearalenone, T-2, and at times fumonisin. There are many derivatives of these mycotoxins that some advocate must also be tested for to broaden the knowledge for troubleshooting. When analyzing the 2009 corn crop, having an expanded mycotoxin analysis (North Dakota Veterinary Diagnostic Laboratory) did not appear to broaden our ability to diagnose or solve a mycotoxin related herd issue. Possibly, the limited number of samples with expanded analysis may have biased this interpretation. Future mycotoxin

challenges may not necessarily support *lesson 8* observations by these authors with broader use of the expanded analysis on a greater number of samples.

#### Lesson 9 – Mycotoxin issues with cows appeared to be mostly digestive upsets, reduced nutrient utilization, and reduced feed efficiency, with some dairies experiencing compromised reproduction and milk production.

The most prominent clinical or acute problem associated with mycotoxins in 2009/2010 was digestive upsets and inconsistent gut health. This would appear as inconsistent manure, loose cows, blood in the manure, and reduced feed digestibility. Milk production swings appeared to follow the gut health and manure as might be expected. Where the epithelia intestinal barrier is known to be a first line of defense against pathogens of all types (Forsberg, 2008), it might not be surprising that the presence of a mycotoxin poison would inflame the gut lining and cause digestive upsets, inconsistent gut health, and loose manure. With inconsistent gut health, it's quite logical that nutrient utilization and feed efficiency would be compromised. Interestingly, feed intake did not seem to be reduced or as variable as one might expect. Although milk production appeared to be compromised in the presence of high levels of fusarium mycotoxins, the impact and decrease seen in milk production was quite variable across herds ranging from very little (<1 - 2 lb/cow/d) to much higher levels (6 - 9 lb/cow/d).

Traditional thinking is that activation of the immune system from *sickness* represents a significant nutritional demand that competes with the productive processes such as milk and protein synthesis. This same line of thinking suggests this drain of nutrients is the cause for decreased performance during sickness (Waldron, 2010). Although there is no doubt that productive efficiency is decreased during sickness, the reason behind this decrease is most likely a coordinated response of metabolic processes dependent upon one another, rather than a competition for substrates. The exact mechanism by which mycotoxins reduce productive efficiency really isn't well understood but likely involves metabolic adaptations beyond the rumen and lower gut health. and may in fact impact the cow at a systemic level much like other poisonous compounds.

## Lesson 10 – Farm necropsy can be a valuable tool to help assess cause of adult cow death.

Necropsy of dead animals to assess and monitor cause of death is often decided to be unnecessary on

dairies. This is in contrast to other livestock management systems including poultry, swine, and feedlots where necropsy is a standard protocol. Necropsy examinations can provide good information, and are particularly valuable if appropriate record systems are used (Garry, 2009). Using necropsy information proved helpful in better understanding where mycotoxins were likely a primary cause of animal health challenges while better understanding other causes of death.

#### Lesson 11- Clays, zeolites, and microbial additives may be beneficial when cows are mycotoxin challenged; while understanding the broad array of products and costs are a challenge in itself.

There are a multiple types of compounds sold, both organic and inorganic by nature, whose chemical and physical properties make them ideal as flow agents and adsorbents (clays, zeolites, mannans). Historically, these compounds have been referred to as *binders;* yet due to regulatory concerns over potential misrepresentation and lack of sound science to support all the products, any claims referring to *mycotoxin binding* is not allowed in the US. Thus, the appropriate term is adsorbents and flow agents for clays and zeolites; while microbial based products are yeast cell wall (**YCW**) derived (mannans) or actual strains of specific naturally occurring live bacteria.

When all possible preventative measures have been taken, the use of adsorbents with some mycotoxin challenges has been shown to be an effective means of mitigating the negative mycotoxin impact: supposedly by binding the mycotoxin rendering it inactive in the animal. Peer reviewed data would suggest that clays and zeolite compounds are more effective with aflatoxin with less binding potential with other mycotoxins. Feeding rate and the type of specific compound appear to be an important consideration. The means by which the glucan fraction of YCW may bind fusarium mycotoxins is not well understood, yet published data has demonstrated in a variety of animal species that YCW may be involved with adsorption of mycotoxins, and is stable throughout the pH range of the digestive tract (Smith, 2010).

This author has fed a number of different commercially available products positioned as beneficial under different mycotoxin and mold challenge conditions with dairy cows. Experiences have been inconsistent, with some very positive outcomes, while other times there were a lack of measureable benefits. The most consistent response

the author saw in the 2009-2010 crop year was with the feeding of a live bacteria to help stabilize gut health while eliminating or reducing the mycotoxin problematic feed ingredients. Of course, solid and consistent feeding management is a prerequisite to getting high levels of performance and productive efficiency, and along with high quality consistent forages, will always trump any feed additive regardless of the additives efficacy. The cost of these mycotoxin and mold related feed additive products often will run at least \$50 - \$150/d on a dairy for every 1000 cows being fed, thus the expense is significant and should be considered in terms of cash flow per month versus cost/cow/d type thinking.

# Lesson 12 – Mother Nature is in control and might only amplify the mycotoxin load that preexists in the soil.

Controlled research and data are lacking to demonstrate a relationship between mycotoxins and tillage and waste feed disposal management practices. However, this is an area that needs more attention and research. With significant minimum tillage practices widely utilized, and with dairies often hauling more spoiled discarded feed to the fields without composting or other treatment; there is discussion on what impact this may have on soil loads of mycotoxins. Practices recommended to minimize field produced molds and mycotoxins include:

- 1) plant hybrids with insect, stalk rot, and ear mold resistance;
- 2) harvest in a timely manner with particular attention to proper moisture levels;
- target corn for silage chopping that is isolated from crops exposed to severe drought or hail damage when possible; and
- consider traditional tillage methods to reduce fungal spore loads in crop residues, particularly where spoiled feed waste was regularly hauled to a field in concentrated locations (Mahanna, 2010).

#### CONCLUSIONS

Feeding the 2009 crop year corn in the Midwest to dairy cows was a challenge due to the high prevalence of primarily fusarium mycotoxins, and in particular DON. Common symptoms included loose cows, inconsistent manure, poor gut health, reduced nutrient utilization, compromised milk production, reduced feed efficiency, and in some cases reduced reproductive performance. Aggressive preventative management practices had to be implemented, which typically added analytical costs to the dairy, and increased feed costs due to ingredient changes being made and/or feed additives being fed to help mitigate the mycotoxin challenge.

The primary strategy used to address and manage the challenge was to better understand and plan the feed sampling protocol, putting a stronger focus on basal ingredients rather than the TMR, and then using the laboratory data to aggressively manage ingredients and ration formulation rather than chasing the problem with feed additives only. The use of certain feed additives did in most cases help mitigate the mycotoxin challenge and served as complementary support to other key feeding management practices. The numerous resources available to support the decision processes and help implement management practices at the farm level were excellent. Credit for these resources largely goes to the university extension service in several states, and several allied industry professionals and companies that worked closely with the dairy extension service.

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