Actlabs Agriculture Services

About Our Company

- Activation Laboratories Ltd. (ACTLABS) was established in 1987 by Dr. Eric Hoffman, an economic geochemist. The laboratory began by providing Instrumental Neutron Activation Analysis (INAA).
- Additional techniques such as ICP-OES, ICP-MS, XRF, AA, etc. added to increase coverage of periodic table and to obtain better detection limits.
- 1994 Global expansion begins.
- 1998 Actlabs becomes one of the first Mineral Lab in the world to obtain ISO 17025 accreditation.
- 2012 Agriculture division has been established and merged with Life Sciences.

Agriculture Mycotoxin Services

Mycotoxins are naturally occurring secondary metabolites of molds and can infect various agricultural commodities in the field and post-harvest.

Actlabs Agriculture offers Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) high-quality testing using a robust method for accurate quantitation of mycotoxins. It prevents overestimation, underestimation, false positives or negatives and can be applied to a wide variety of matrices.

Mycotoxins – Why Are They Important?

Mycotoxins pose a serious health hazard to both humans and animals:

- In animals, ranging from simple feed refusal all the way to death
- In humans, similar effects where many are potent carcinogens
- Can travel up food chain as they are not typically removed by processing

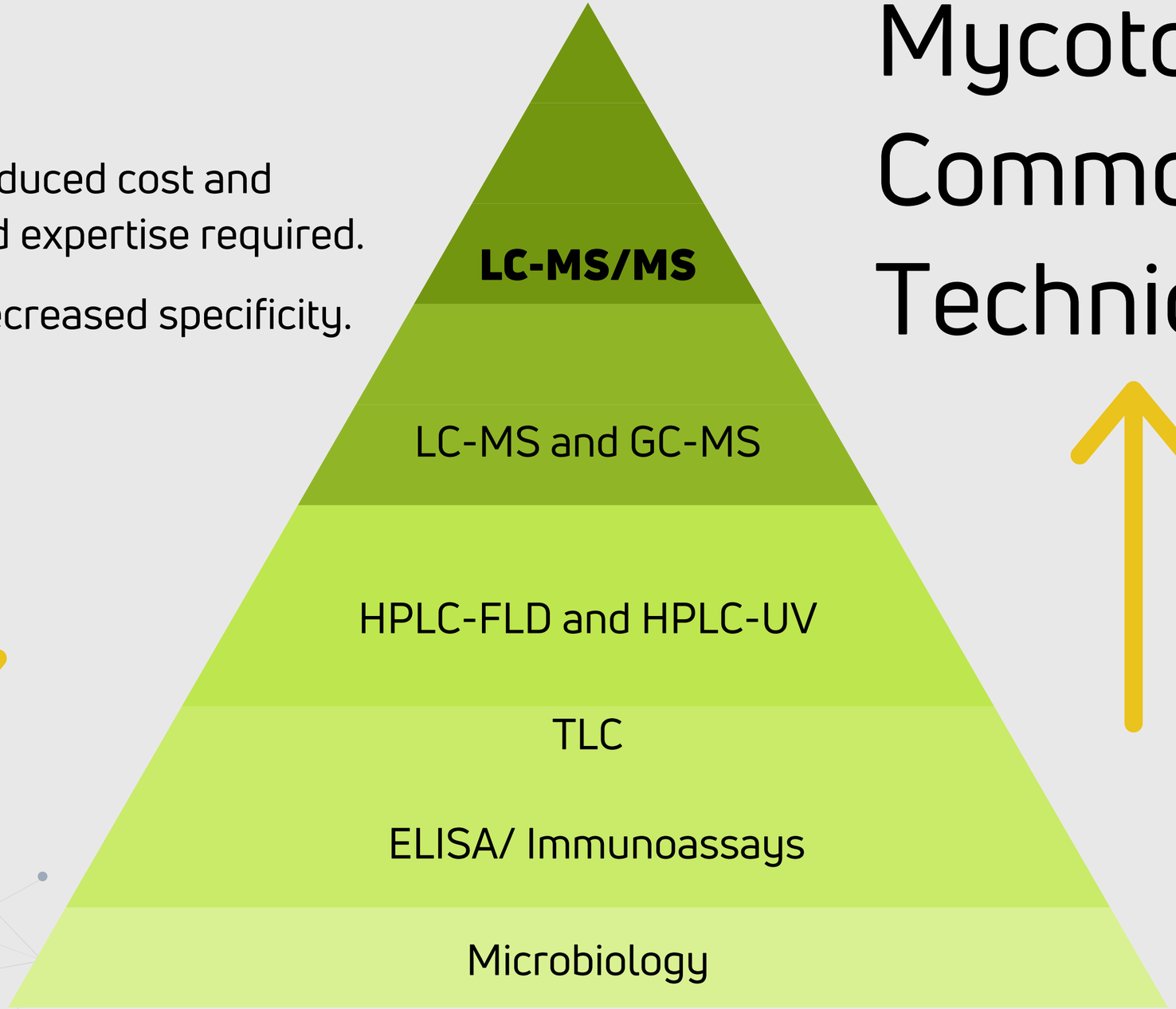
Mycotoxins pose an economic impact:

- Reduced crop yields
- Spoiling of harvested and processed crops
- Reduced animal performance

Mycotoxins – Common Analytical Techniques

Pro: Reduced cost and reduced expertise required.

Con: Decreased specificity.

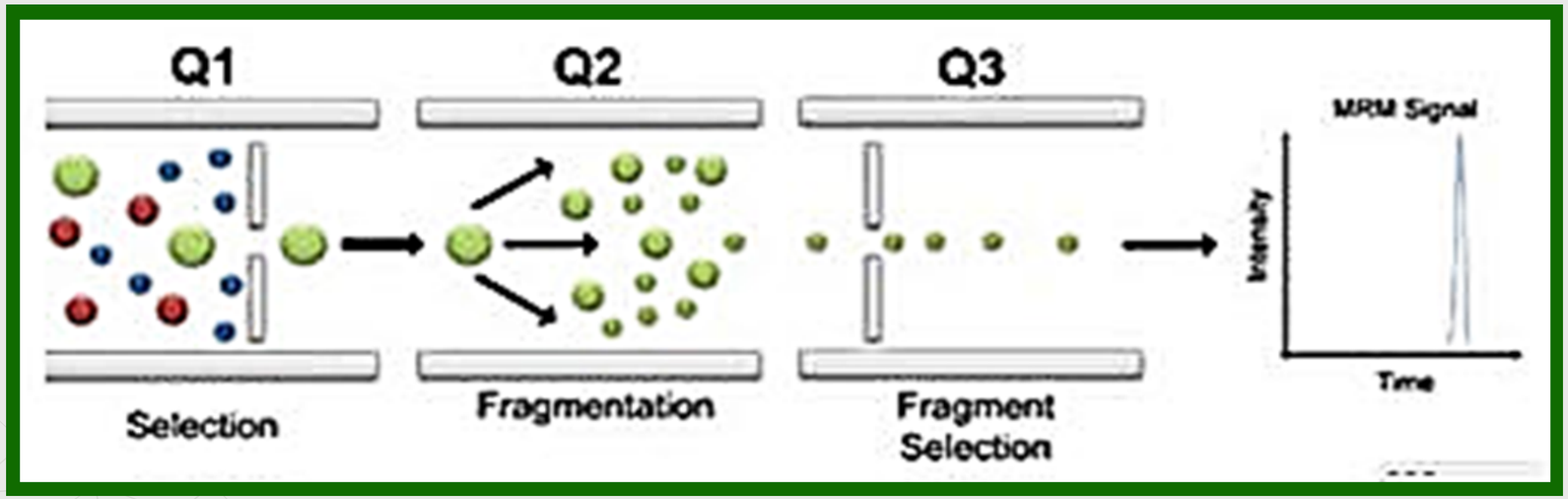


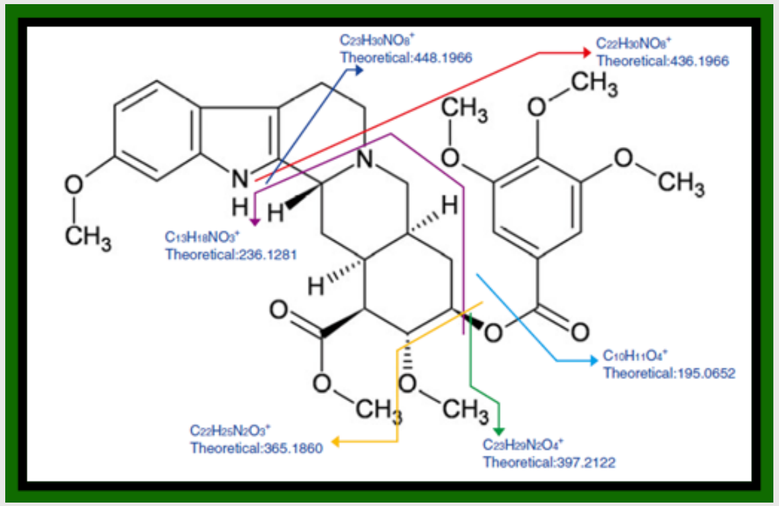
Pro: Increased specificity.

Con: Increased cost and increased expertise.

Actlabs LC-MS/MS Mycotoxins Analysis

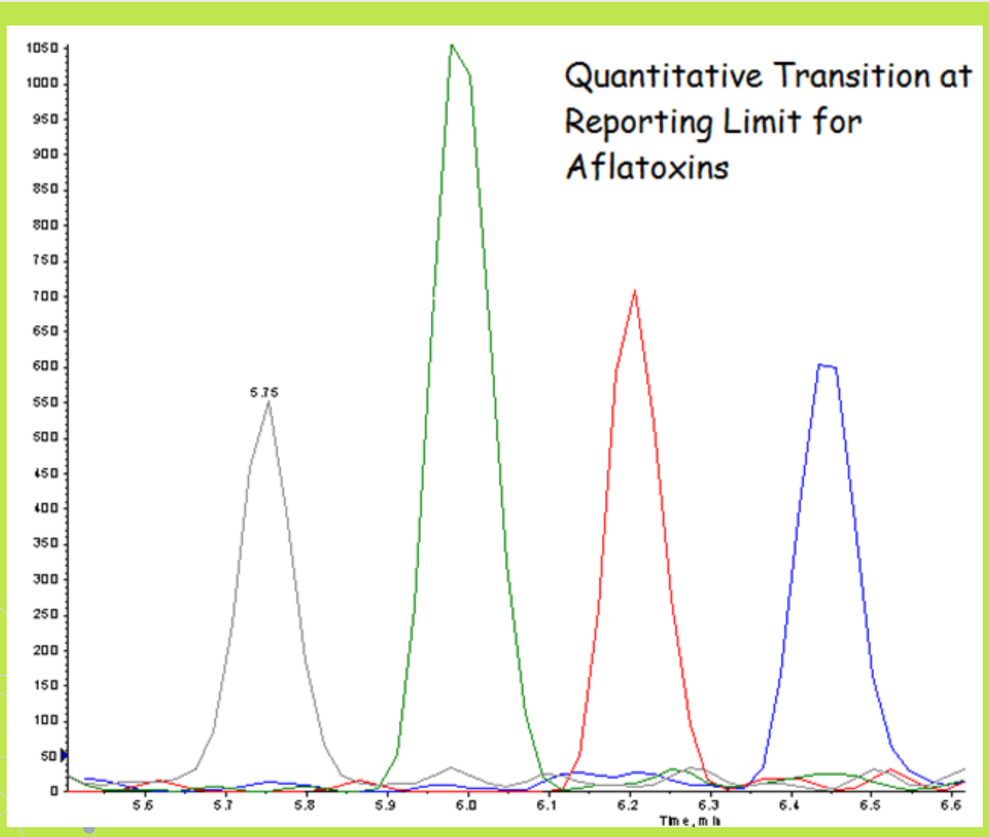
- Liquid Chromatography Tandem Mass Spectrometry (LCMSMS) uses HPLC separation and molecular weight for detection.
- Instrumentation and level of training is significantly higher than HPLC.

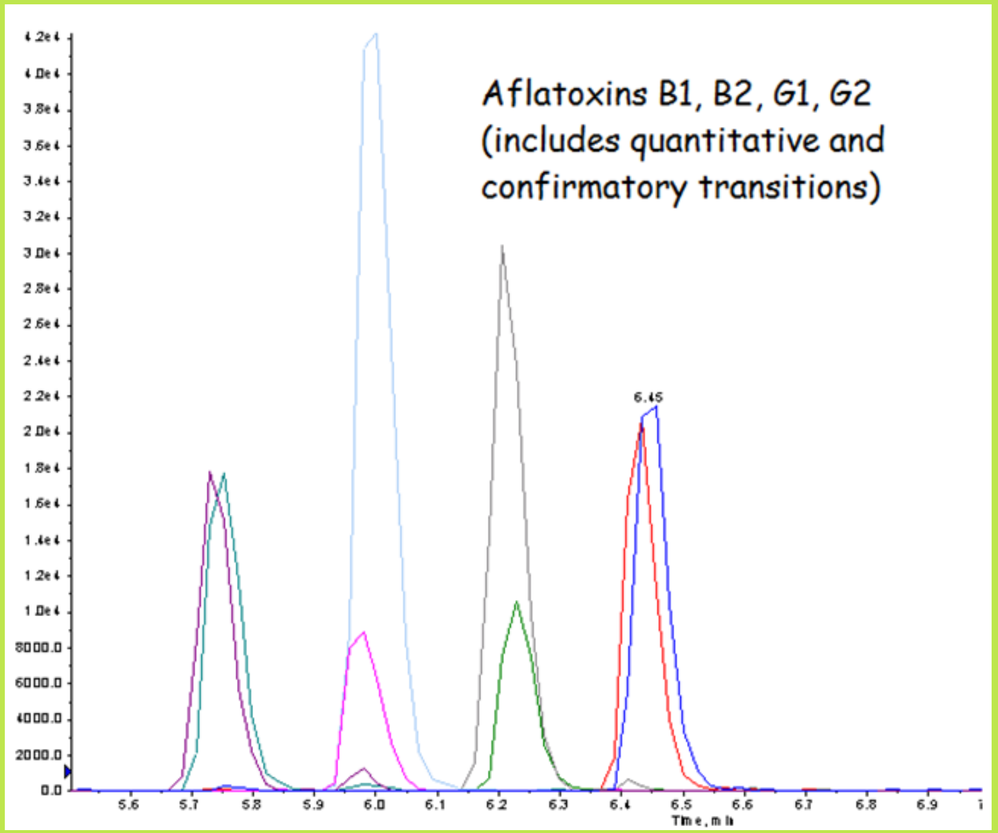




Actlabs LC-MS/MS Mycotoxins Analysis

- We have included an additional MRM transition for greater specificity when required
- Excellent signal-to-noise seen around reporting limit





Our 16 Mycotoxins Screen Includes

- Aflatoxin B1
- Aflatoxin B2
- Aflatoxin G1
- Aflatoxin G2
- Deoxynivalenol (DON)
- 3 -Acetyl-deoxynivalenol
- 15-Acetyl-deoxynivalenol
- Diacetoxyscirpenol (DAS)
- Fumonisin B1
- Fumonisin B2
- Ochratoxin A
- Sterigmatocystin (Sterig)
- T-2
- HT-2
- Zearalenone
- Mycophenolic acid

Hilyses®
The Bionic Prebiotic

USA USA - Mycotoxins Risk assessment

Sample description:	**Farms Pen 3 TMR 080223**
Submitted for:	

	Sample reference	**Sample ID: X23-001488**
	Mycotoxins (ppb)	
1	Aflatoxin B1, B2, G1, G2	
2	Deoxynivalenol (DON)	**2,860**
3	3-acetyldeoxynivalenol	
4	15-acetyldeoxynivalenol	
5	Fumonisin B1, B2	**100**
6	Ochratoxin A	
7	T-2 toxin	
8	HT-2 toxin	**130**
9	Diacetoxyscirpenol	
10	Sterigmatocystin	
11	Zearalenone	**80**
12	Mycophenolic Acid (MPA)	

Number of toxins found	**4**
Risk Level	
NOVIN S feed rate (gr/head per day)	**28**
NOVIN S feed rate (lbs/hd/day)	**.06**

Hilyses feed rate (gr/head per day)	**14**
Hilyses feed rate (lbs/hd/day)	**.03**

Geobond (oz/hd/day)	**2**
Geobond (lbs/hd/day)	**.125**

Low
Medium
High

Dairy Risk Management Assessment August 2023

- Main observation is that the level of contamination is high
- Sample has multiple toxin contamination representing a remarkably high risk
- Sample is contaminated with DON, Zearalenone, HT-2and with some trace of Fumonisins.
- 5 different toxins, which will result in synergistic impact on metabolism.
- In vitro studies showed an additive effect in the toxicity when ZEN and DON are co-occurring in the feed (Ren et al., 2015).
- No Aflatoxin
- If cows are exhibiting outward signs of stress, add NovinS at one ounce for two weeks then reduce
- Sample is very problematic- Find biggest toxin contributor and eliminate or reduce usage. Dilution is the best solution
- Most toxins are of non-polar form, so cannot be bound with simple clays only

Deoxynivalenol (DON):

- There will be an impact on gut integrity and intestinal barrier due to the presence of DON. DON presence impairs the ruminal fermentation and decreases the quantity of available protein in the duodenum (Danicke et al., 2005).
- Deoxynivalenol (DON): In ruminants DON is almost completely transformed (>99%) to the less toxic de-epoxidized metabolite DOM-1 by the ruminal microorganisms. However, the use of feed additives could reduce the number of microorganisms, specifically Protozoa. Protozoa are a ruminant's natural defense against DON, consequently cows could be more sensitive to DON presence

- Acidosis increases toxicity: a pH>5.2 inhibits the complete transformation of DON to DOM-1. So, calves or adults with previous history of ruminal acidosis may have less effective de-epoxidation. Then, they could be more susceptible to the toxic effects of DON which mainly are leukocyte apoptosis, alterations in the immune system and damage in the intestinal tissues
- DON is a common mycotoxin in cereal grains and their plants, but the concentration found in the analyzed maize is considerably higher than the common concentration levels.
- Milk production: it is not clear the impact of DON in milk production however some studies pointed out that DON concentrations in the feed (>2.6ppm) could cause a reduction in milk production (13%) (Charmley et al. 1993).
- The conjugated DONs, 3-A and 15-A contain more double and triple bonds to release more free radicals increasing oxidative stress
- The DON Derivative - 15-acetyldeoxynivalenol is usually found where expelled cuds are present
- Immune system: DON intake has been linked with the alteration of the immune function in dairy cattle (Korosteleva et al., 2009).

Fumonisins (FBs)

- Fumonisins (FBs): FBs are very common in corn; they are hardly absorbed by animals, especially ruminants but their presence is of concern because they are highly toxic.
- Liver changes: exposure to FBs has been linked to an impairment of liver and possibly kidney function (Mathur et al., 2001). Chronic FBs exposure at lower levels in ruminants showed an increase of aspartate aminotransferase levels.
- Immune response is reduced: FBs presence impairs lymphocyte blastogenesis (Osweiler et al., 1993).
- Oxidative stress: FBs cause oxidative stress in the cells from different organs (liver, kidney, lungs, …) (Dassi et al., 2018).
- Not excreted in the milk: as Fbs are poorly absorbed, there is a minimal FBs (~0.05%) carry over into milk (Voss et al., 2007).

- AF+FBs: co-occurrence of aflatoxins and Fumonisins can determine an important delay in the reproductive career (Abeni et al., 2014).

Zearalenone (ZEN)

- Zearalenone - ZEN has a chemical structure similar to estrogen and can produce an estrogenic response in animal, several case reports have related ZEN to estrogenic responses in ruminants. (Khamis et al., 1986)
- ZEN contaminations can result in cystic ovaries, anestrus, and early embryonic death
- Zearalenone is highly estrogenic due to its affinity to the estrogen receptor.
- Zearalenone is rapidly absorbed by the gastrointestinal tract, with absorption rates up to 80-85%
- Fertility rate is reduced: ZEN presence has been widely linked to a reduction in the conception rate in cows. Fertility alterations can be present from a low ZEN concentration (50ppb). ZEN is a powerful estrogenic compound which can alter the estrus cycles. There is a significant decrease in the maturation rates of oocytes exposed to ZEN (Minervini et al., 2008).
- Oxidative stress: several in vitro studies showed ZEN induces oxidative stress in the cell and inhibits protein synthesis (Tatay et al., 2017).
- Hepatotoxic: even low concentrations of ZEN can produce toxicity in the liver cells causing the apparition of hepatocellular adenomas (Sun et al., 2014).
- Immunotoxicity: ZEN presence resulted in alterations to the immunological parameters such as the inhibition of lymphocyte proliferation (Vlata et al., 2006).
- Heifer Development: The clinical effects observed on heifers vary somewhat from cows. Mammary gland enlargement, swollen vulvas, and vaginitis are frequently observed more often in heifers as compared to cows. After being fed a ration containing moldy corn, 17 of 20 prepubertal dairy heifers developed enlarged mammary glands in at least one quarter.

- Note: In vitro studies showed an additive effect in the toxicity when ZEN and DON are co-occurring in the feed (Ren et al., 2015)

T-2 & HT-2

- The HT-2 is most concerning- recommend testing individual feed ingredients to discover cause. Whole Cotton Seed included in TMR?
- The HT-2 is a Macrocyclic trichothecenes. These compounds vary in their toxicity and are suspected immunotoxins. **HT-2 is a metabolite from T-2:** but during digestion T-2 is fast converted to HT-2. So, the toxic effect of T-2 and HT-2 cannot be differentiated. T-2 and HT-2 are *Fusarium* mycotoxins like DON; however, T-2 toxins toxicity are much higher than DON toxicity
- **Delay in the ovulation**: a chronic exposure of 0.025 mg T-2 toxin/kg bodyweight for more than 20 days in cows, caused a 2-day delay in the ovulation. Progesterone level was also lower because of T-2 exposure.
- **Immune response is reduced**: total protein, albumin and immunoglobulin fractions were decreased in the calves chronically exposed to T-2 toxin. T-2 toxins are well-known to cause alimentary toxic aleukia.

- **Oxidative stress:** T-2 toxins induce oxidative stress, causing DNA damage, apoptosis, inhibiting protein synthesis and damaging lipids.
- The trichothecenes are an exceptionally large family of chemically related mycotoxins produced by molds like Fusarium, Trichoderma, Trichothecium and others. T-2 / HT-2 Toxin belongs to the group of type A trichothecenes. In addition, there are type B trichothecenes, the most important of which being Deoxynivalenol (DON).

NovinS

- A multi-functional preventive approach to counteract stress in the presence of toxic contaminants and guarantee high performance in today's challenging production conditions. NovinS® expands beyond traditional binding

approaches of clays and yeast cell wall products. NovinS supports natural detoxification. NovinS® with five modes of action.

- NovinS® - Scientifically proven modes of action:
- Liver and kidney support to reduce inflammation
- The selected plant extracts are proven to support the function of these organs when confronted with toxic stressors (e.g., toxin blockade at membrane level, protein synthesis enhancement, antibiotic activity, anti-inflammatory effect etc.). Liver & kidney detoxification remains a key target to animal performance.
- Preventing Oxidative stress
- Counteracting immune suppression and strengthening the animal's natural immune responses
- Enhancing natural detoxification processes
- Binding of water soluble (polar) toxins reduces their bioavailability.

Hilyses

- Hilyses @ 38% protein can replace any yeast culture in the ration
- In Vitro studies show that Hilyses binds >80% of Zearalenone
- Hilyses contains 26% β-1,3 and β-1,6-Glucans. β-1,3 and β-1,6-Glucans are well documented for binding mycotoxins
- Hilyses contains 17% mannan and has excellent agglutination data for Salmonella, E coli and Clostridium bacteria
- Protection with NovinS and Hilyses is strongly recommended at the above feeding rates
- Effects of mycotoxins vary depending on age, external environment, and additional stress.
- Consult your MarSyt Animal Feed Specialist for further details

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Mycotoxin Analysis
Analytical Report

Actlabs Agriculture

Report ID: US23-D-AUG9-001
Received Date: August 09, 2023
Printed Date: August 16, 2023

Innov ad NV/SA
Marsyt Inc.
54 Brown Street
Elizabethtown, Pennsylvania 17022

Sample ID: X23-001488
Sample Number: #1
Sample Description: Pen 3 TMR 080223
Submitted For:

Mycotoxin	Value (Dry Basis)	Units	RL	Method ID
Aflatoxin B1	< 1	ppb	1	AGR DI M 1.3
Aflatoxin B2	< 1	ppb	1	AGR DI M 1.3
Aflatoxin G1	< 1	ppb	1	AGR DI M 1.3
Aflatoxin G2	< 1	ppb	1	AGR DI M 1.3
Deoxynivalenol (DON)	**2.86**	**ppm**	0.06	AGR DI M 1.3
3-Acetyl-Deoxynivalenol	< 0.06	ppm	0.06	AGR DI M 1.3
15-Acetyl-Deoxynivalenol	< 0.06	ppm	0.06	AGR DI M 1.3
Fumonisin B1	**0.1**	**ppm**	0.1	AGR DI M 1.3
Fumonisin B2	< 0.1	ppm	0.1	AGR DI M 1.3
Ochratoxin A	< 0.003	ppm	0.003	AGR DI M 1.3
T-2	< 0.06	ppm	0.06	AGR DI M 1.3
HT-2	**0.13**	**ppm**	0.06	AGR DI M 1.3
Zearalenone	**0.08**	**ppm**	0.03	AGR DI M 1.3
Diacetoxyscirpenol	< 0.06	ppm	0.06	AGR DI M 1.3
Sterigmatocystin	< 0.03	ppm	0.03	AGR DI M 1.3
Mycophenolic Acid	< 0.03	ppm	0.03	AGR DI M 1.3

Results Approved By:

Carolyn Fraser, Manager of Agriculture Services