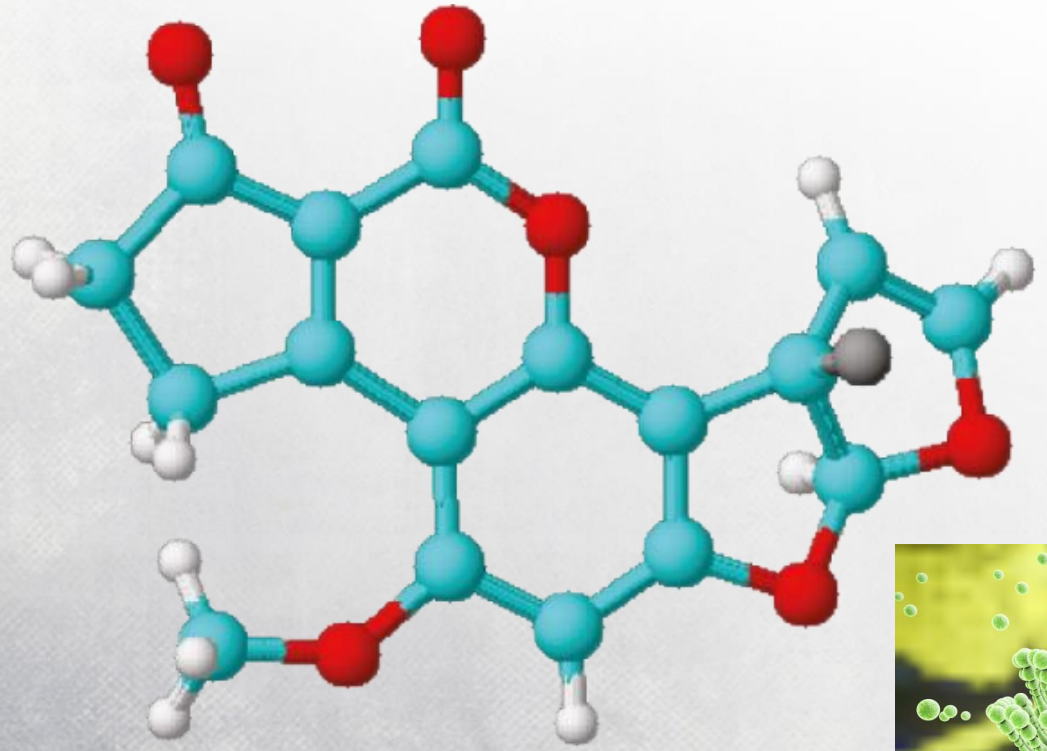
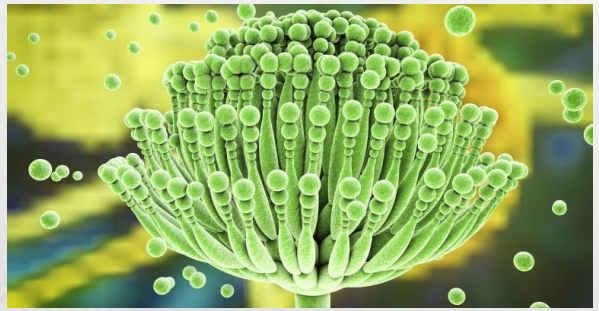
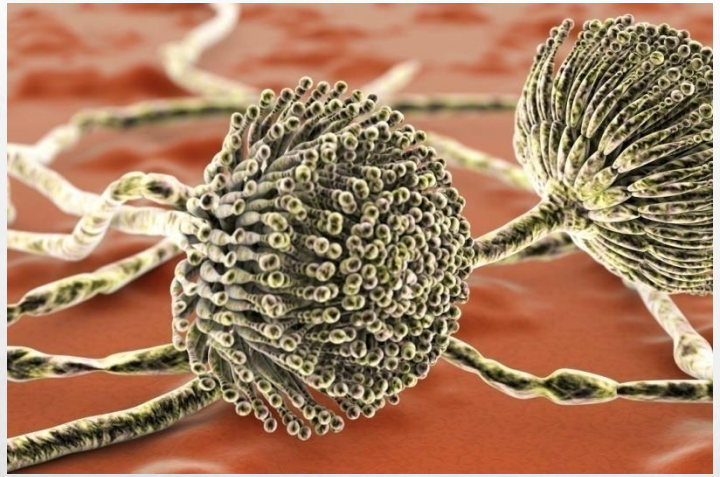


Aflatoxin M1 : Role of Compound Feed

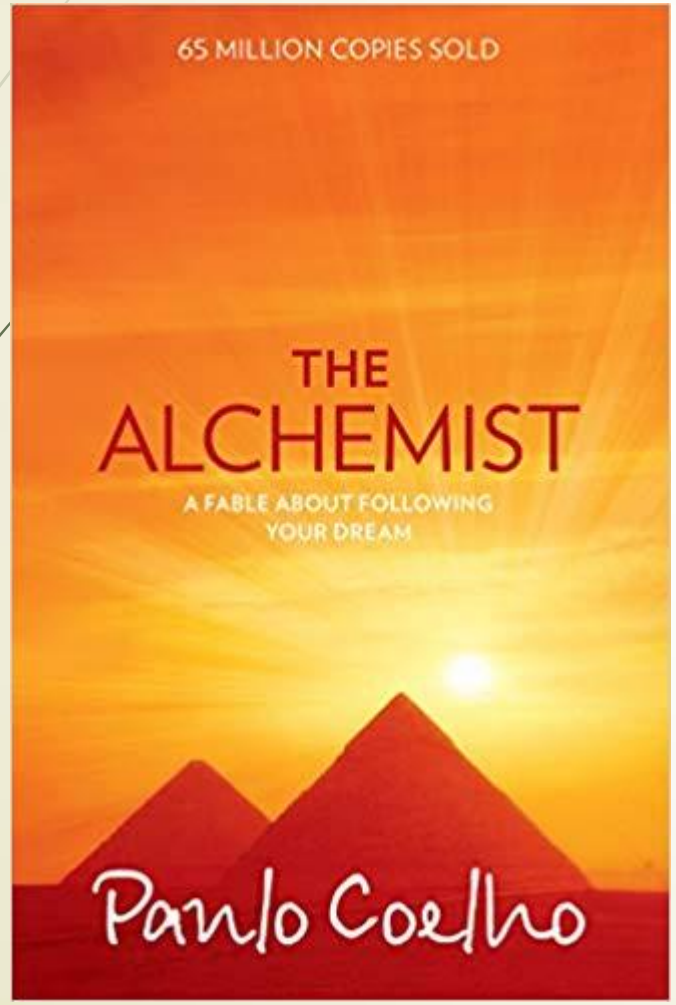


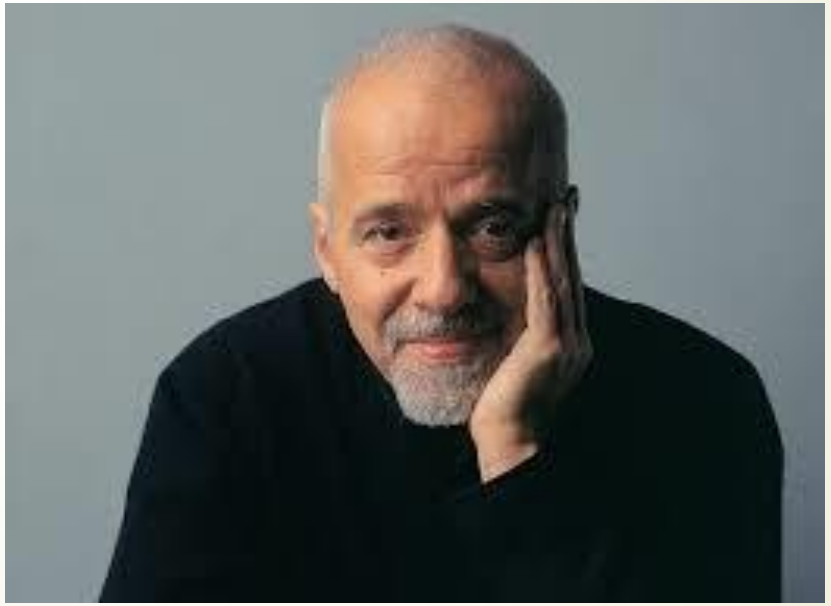


- Seeking Simple Solutions to a Complex Problem

VIZIVA SERVICES

When you want something, all the universe conspires in helping you to achieve it





VIZIVA
SERVICES

Aflatoxins & Other Mycotoxins

Fungi

Requirements for Fungal Growth

- Moisture
- Temperature
- Nutrients
- pH

Controlling Quality of Incoming Material

- Existing Fungal Growth
- Prevention of further growth

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Controlling Quality of Incoming Material

- Existing fungal
- Visible
- Invisible

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Visible Fungal growth

- No correlation between visible fungal growth and mycotoxins levels

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Controlling further fungal growth

- **Moisture** ✓
- ~~Temperature~~
- ~~Nutrients~~
- ~~pH~~

VIZIVA SERVICES

Aflatoxins Levels

- Fungal growth & therefore, mycotoxins occur in pockets
- Thorough sampling is extremely important

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Aflatoxin Levels

- Based on past experience and industry surveys, we need to identify critical RMs

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Critical Ingredients

- Fungus grows on surface
- Material offering more than one surface

Alltech's Mycotoxins Survey – South Asia - 2011

Sample Details

- Number of samples: 176
- Collection Period: May to Nov 2011
- Major samples: corn, bajra (pearl millet), soybean meal, groundnut cake, broiler feed, breeder feed, layer feed, cattle feed, jowar fodder
- Others: rice, rice/wheat bran, distillers byproducts, cottonseed cake, till cake, sorghum, and chunni
- If number of samples more than 5, detailed data analysis was conducted

Alltech® ...naturally

Sample Distribution

Sample Type	Number of Samples
Maize	40
Broiler Feed	29
Soybean meal	20
Breeder Feed	15
Compound Cattle Feed	8
Jowar fodder	7
Layer Feed	6
Bajra	5
Groundnut cake	5
Others	41

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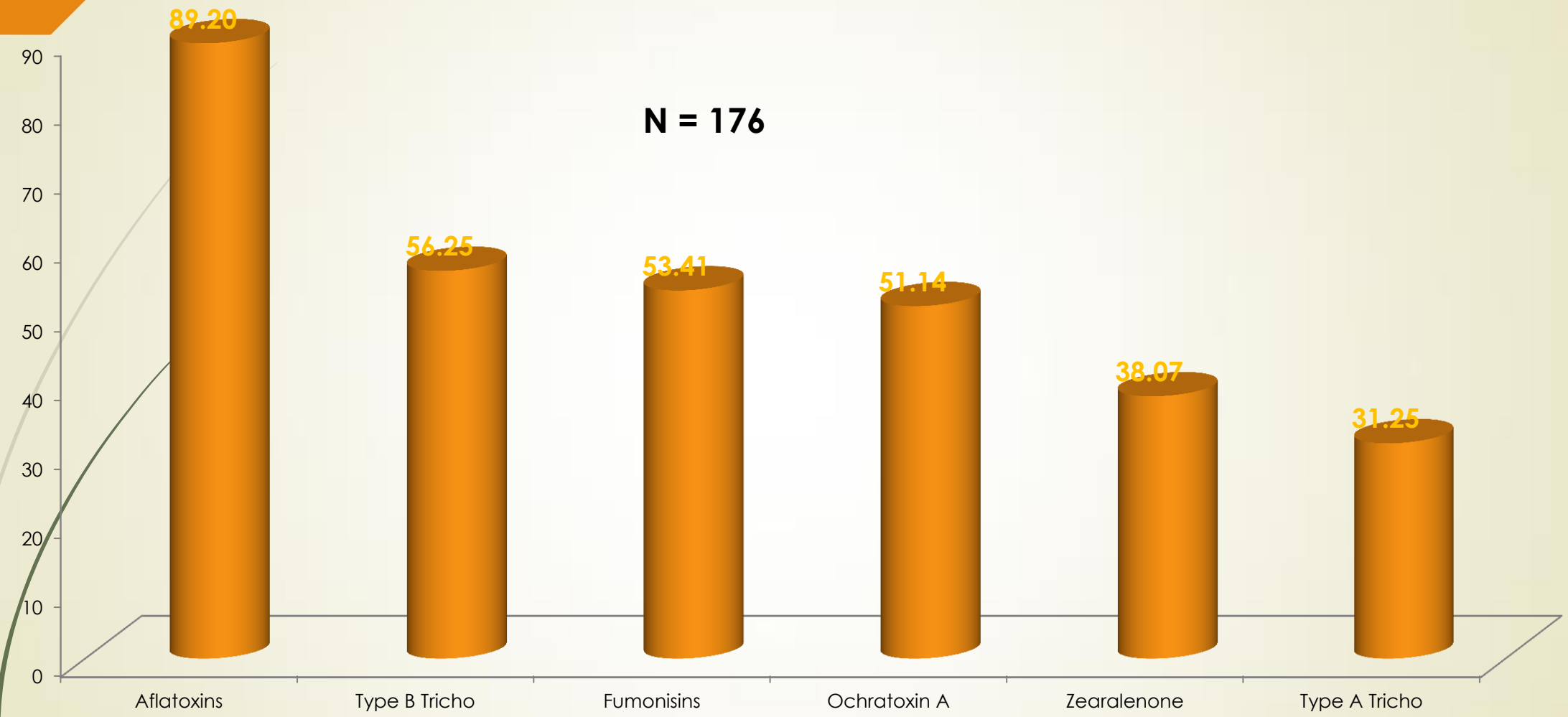
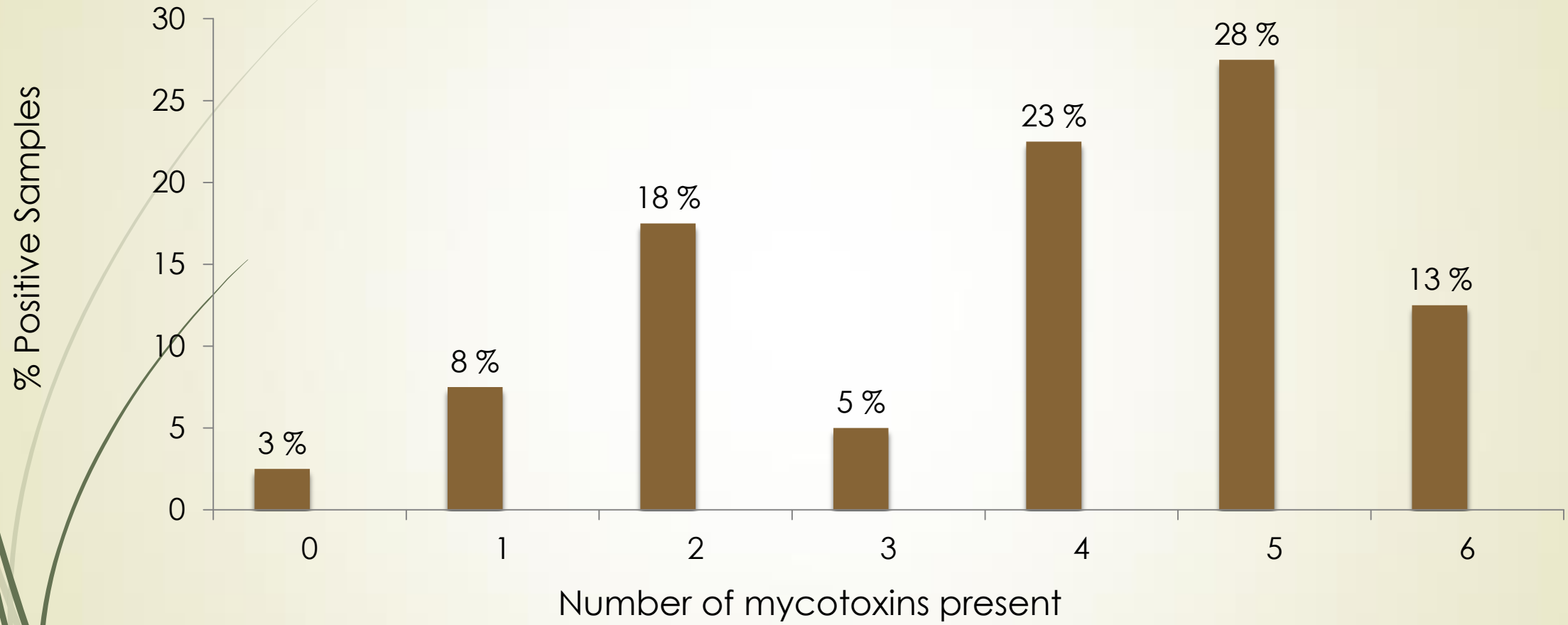
Percentage of Samples Positive for Mycotoxins



Multiple Mycotoxins in Maize (N=40)



BIOMIN

World Mycotoxin Survey

The Global Threat

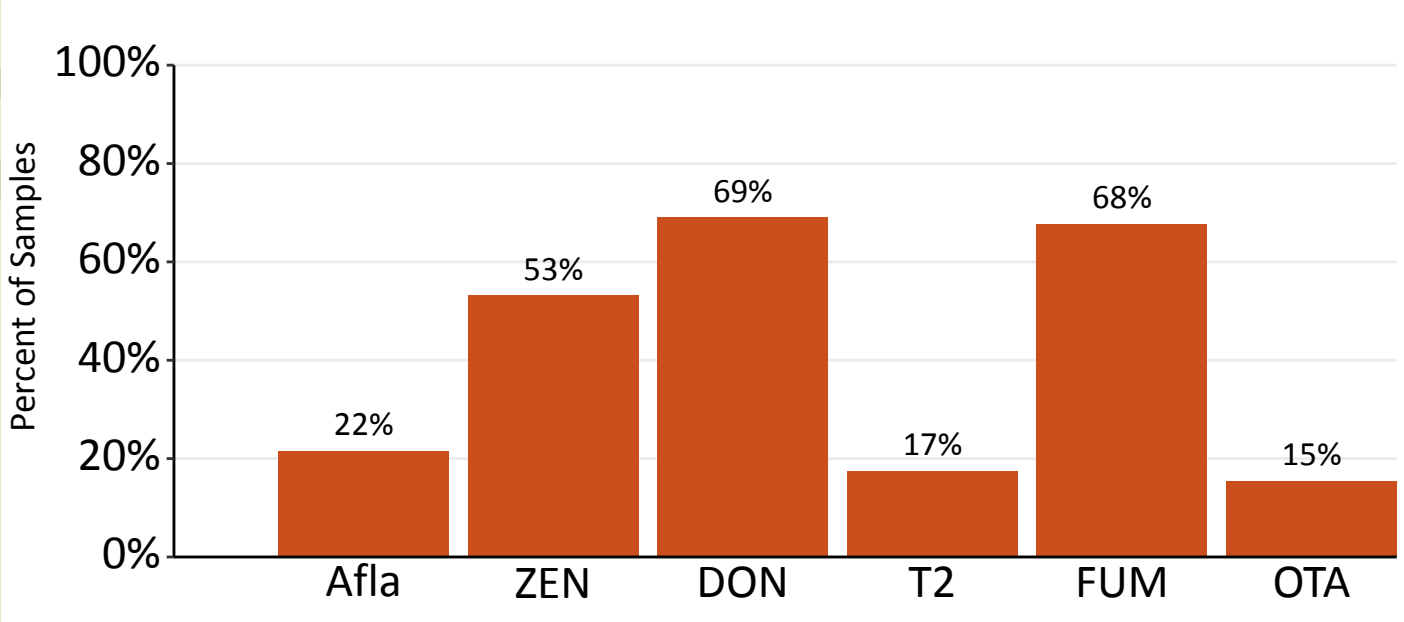
January to September 2018

Biomin®

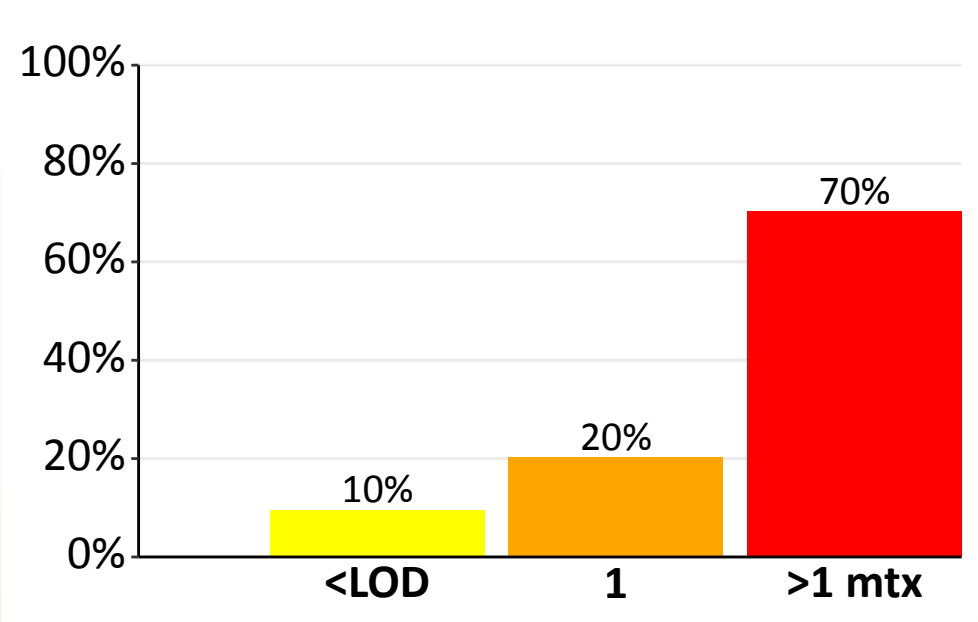
Global Report Jan 2018 to Sep 2018

Total Risk Level: 67%*	Afla	ZEN	DON	T2	FUM	OTA
Number of samples tested	11055	11343	11236	7992	10628	6815
% Contaminated samples	22%	53%	69%	17%	68%	15%
% Above risk threshold	16%	22%	55%	3%	40%	2%
Average of positive (ppb)	19	125	796	68	1611	13
Median of positive (ppb)	4	39	396	24	680	2
Maximum (ppb)	4890	10790	53796	6062	123444	5912

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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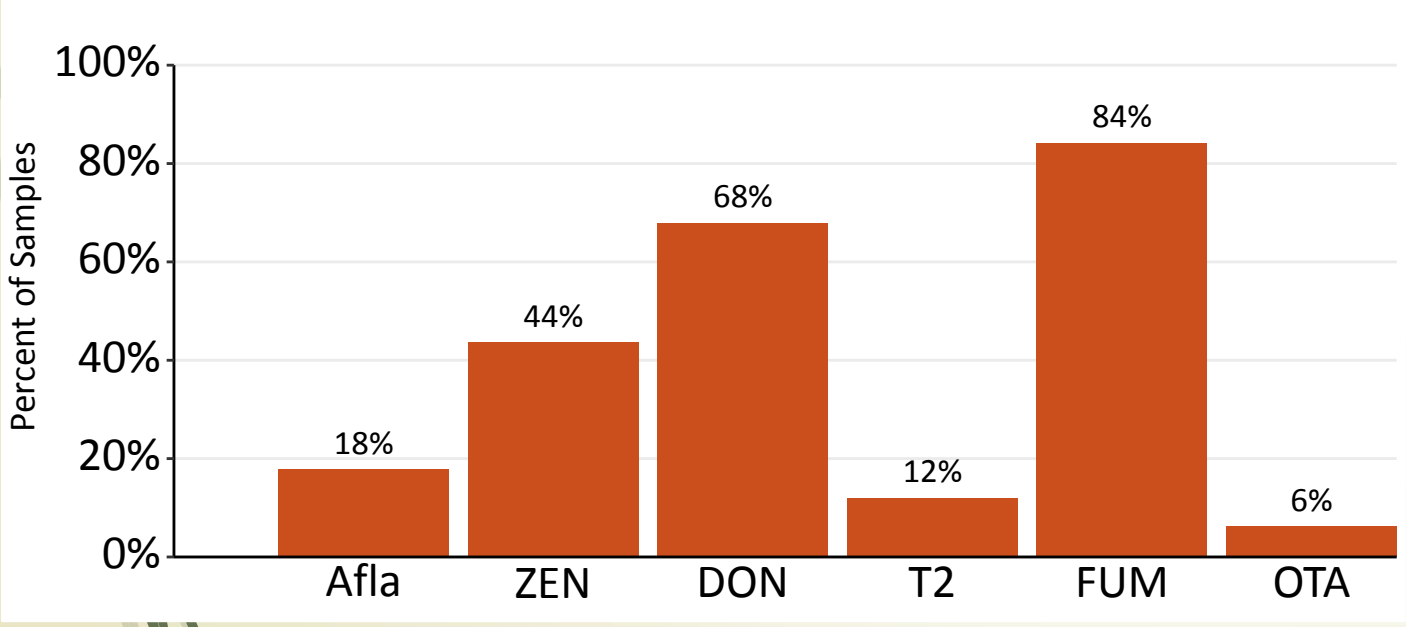
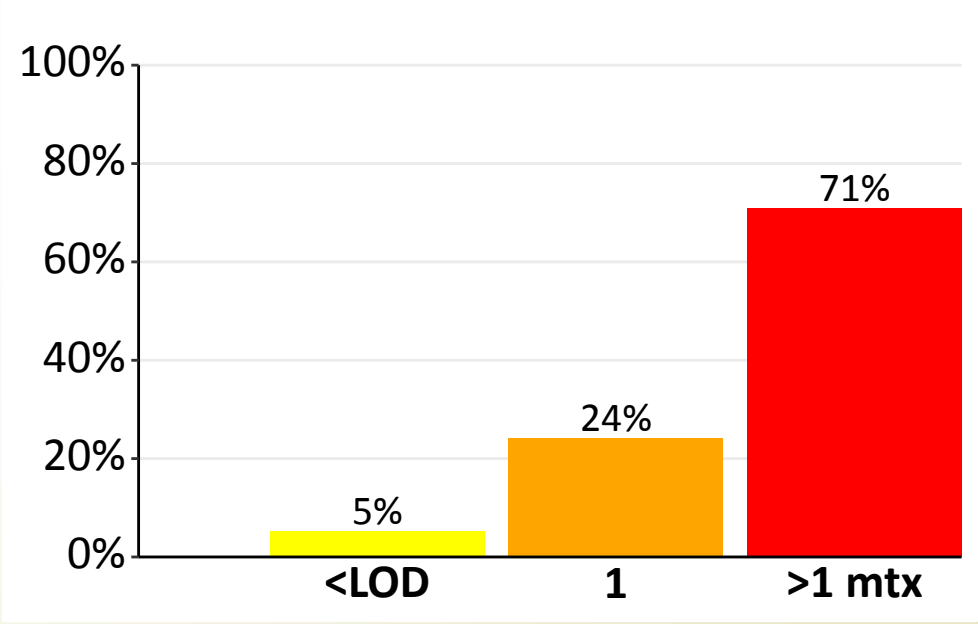
Corn (Kernels) Jan 2018 to Sep 2018

Total Risk Level: 75%*	Afla	ZEN	DON	T2	FUM	OTA
Number of samples tested	3974	3476	3071	1683	3487	1233
% Contaminated samples	18%	44%	68%	12%	84%	6%
% Above risk threshold	15%	22%	63%	4%	66%	1%
Average of positive (ppb)	18	157	646	186	2360	96
Median of positive (ppb)	4	51	430	37	1134	3
Maximum (ppb)	605	5087	40700	6062	47485	5912

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample

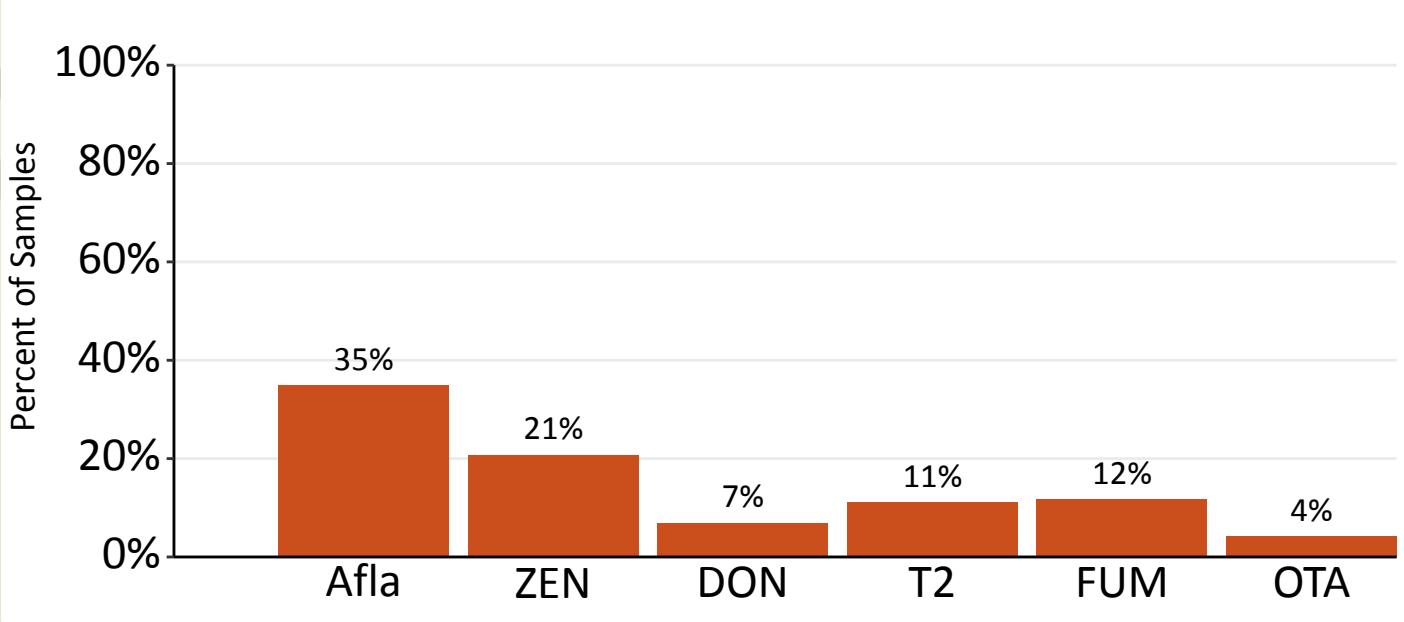


Biomin®

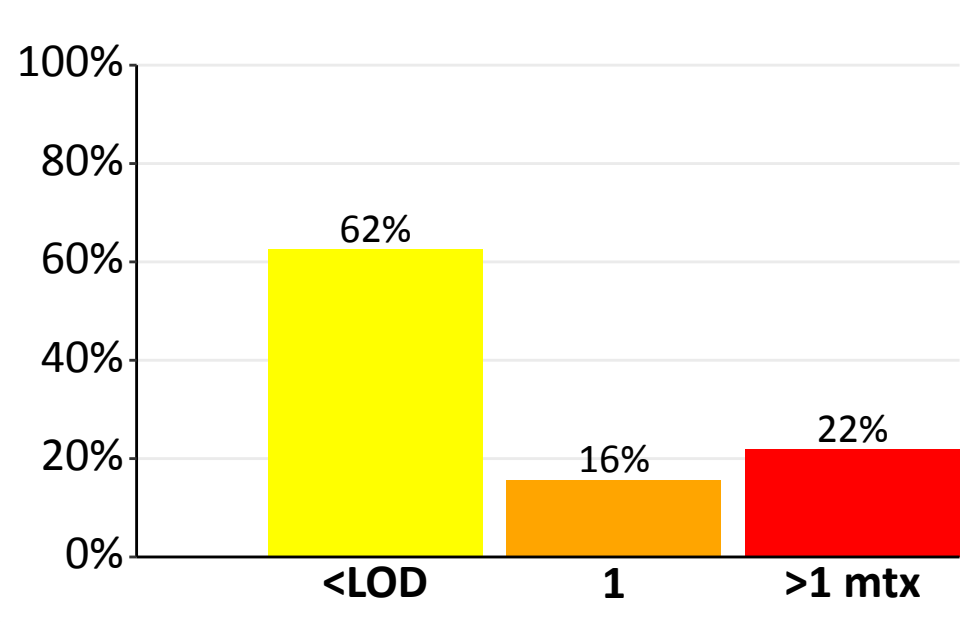
Sorghum Jan 2018 to Sep 2018

Total Risk Level: 36%*	Afla	ZEN	DON	T2	FUM	OTA
Number of samples tested	172	29	29	27	43	24
% Contaminated samples	35%	21%	7%	11%	12%	4%
% Above risk threshold	35%	17%	3%	0%	0%	0%
Average of positive (ppb)	5	172	270	24	95	3
Median of positive (ppb)	5	160	270	25	61	3
Maximum (ppb)	40	329	520	25	197	3

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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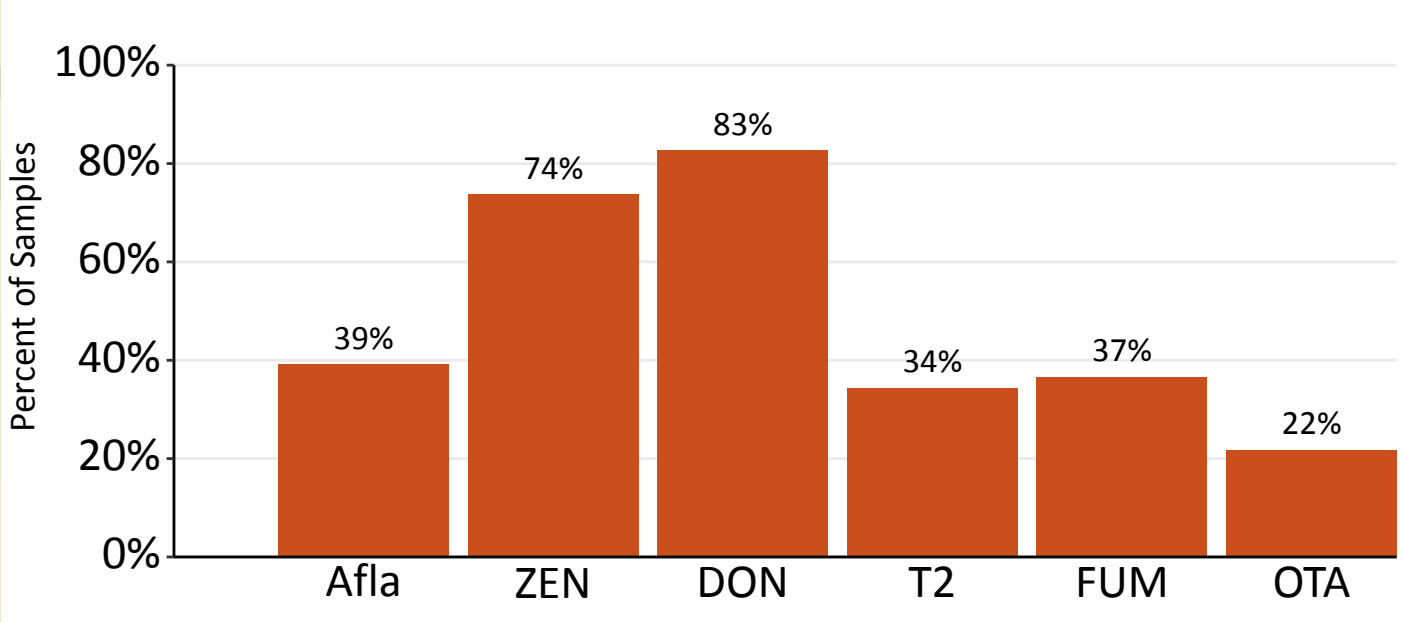
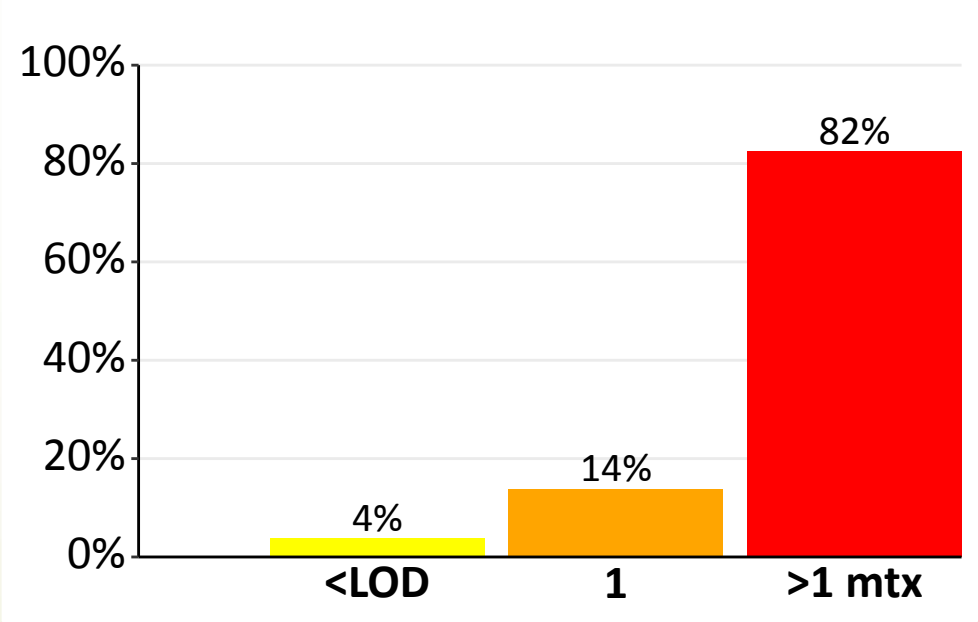
Wheat Bran Jan 2018 to Sep 2018

Total Risk Level: 92%*	Afla	ZEN	DON	T2	FUM	OTA
Number of samples tested	355	346	368	303	361	161
% Contaminated samples	39%	74%	83%	34%	37%	22%
% Above risk threshold	37%	45%	79%	6%	4%	2%
Average of positive (ppb)	5	139	2352	63	335	4
Median of positive (ppb)	4	68	1324	35	270	2
Maximum (ppb)	68	2067	24880	583	6050	27

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample

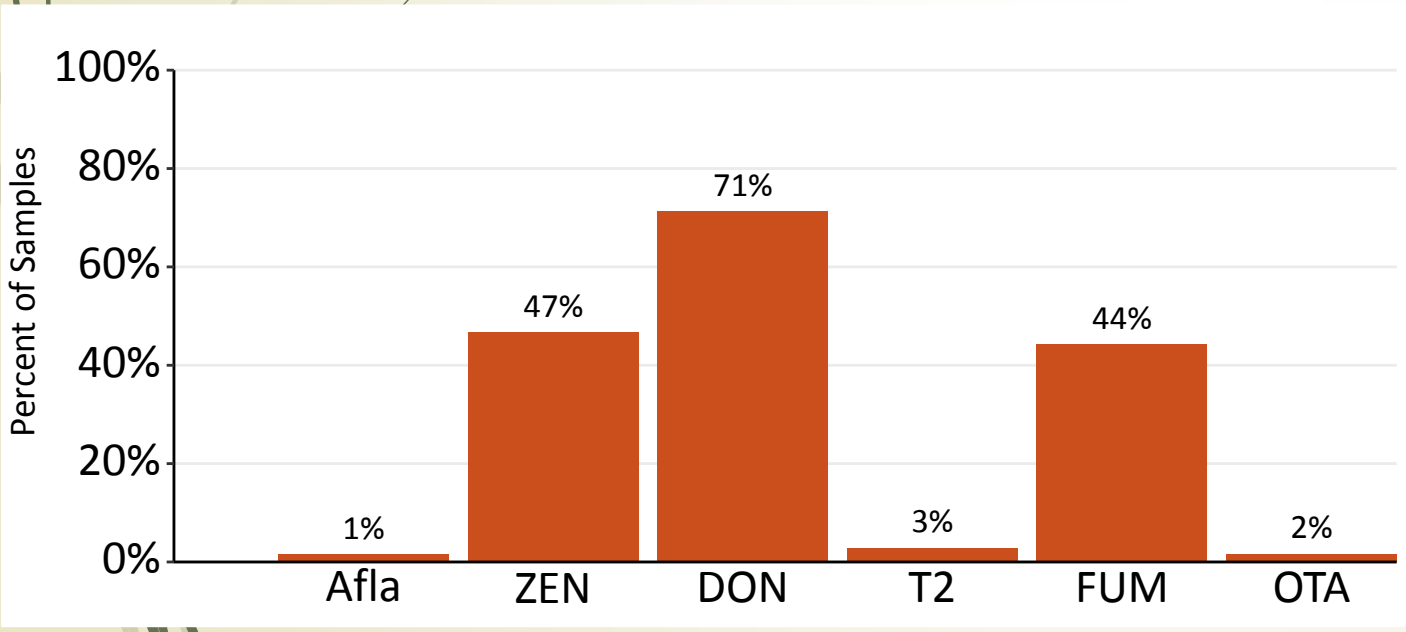


Biomin®

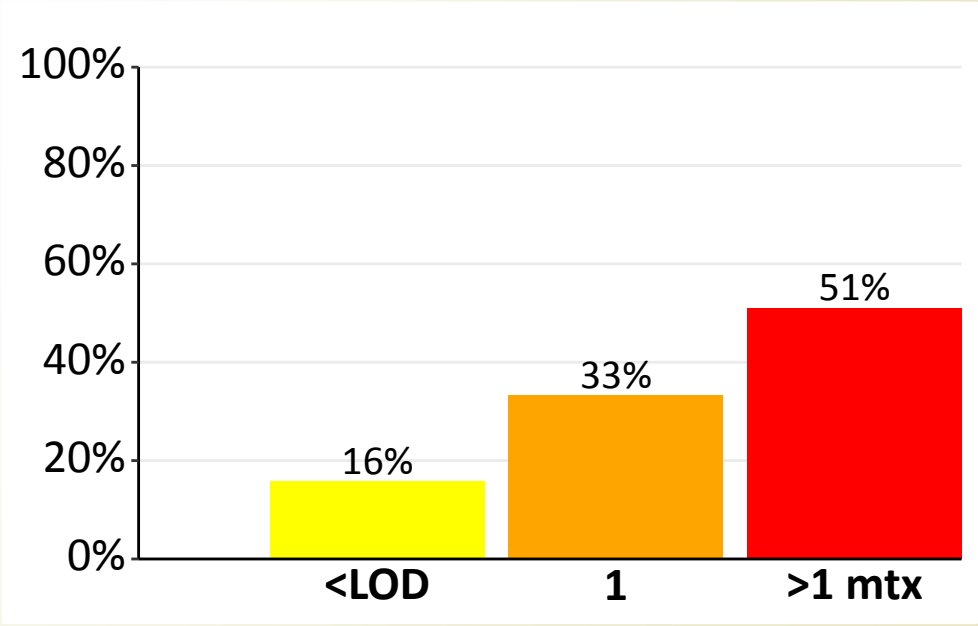
Corn Silage Jan 2018 to Sep 2018

Total Risk Level: 64%*	Afla	ZEN	DON	T2	FUM	OTA
Number of samples tested	491	504	508	495	493	492
% Contaminated samples	1%	47%	71%	3%	44%	2%
% Above risk threshold	1%	27%	56%	1%	14%	0%
Average of positive (ppb)	4	192	756	25	1006	15
Median of positive (ppb)	3	62	402	4	183	4
Maximum (ppb)	10	3155	11000	110	70418	100

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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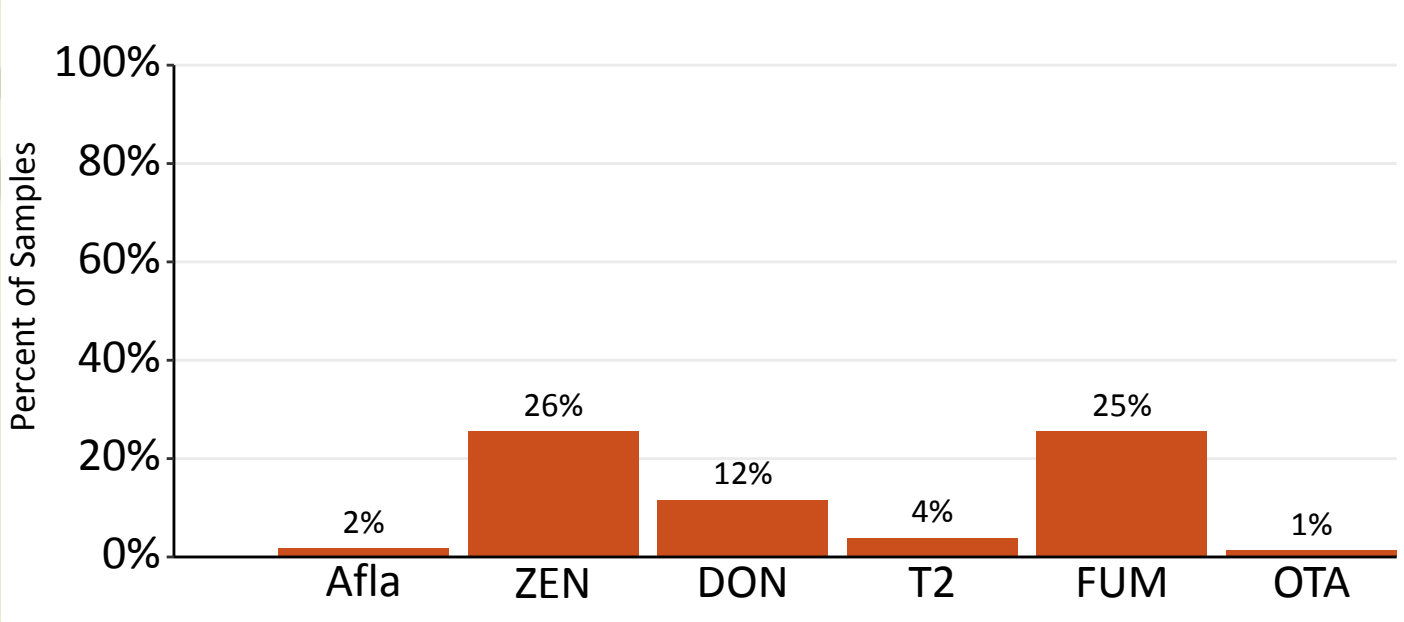
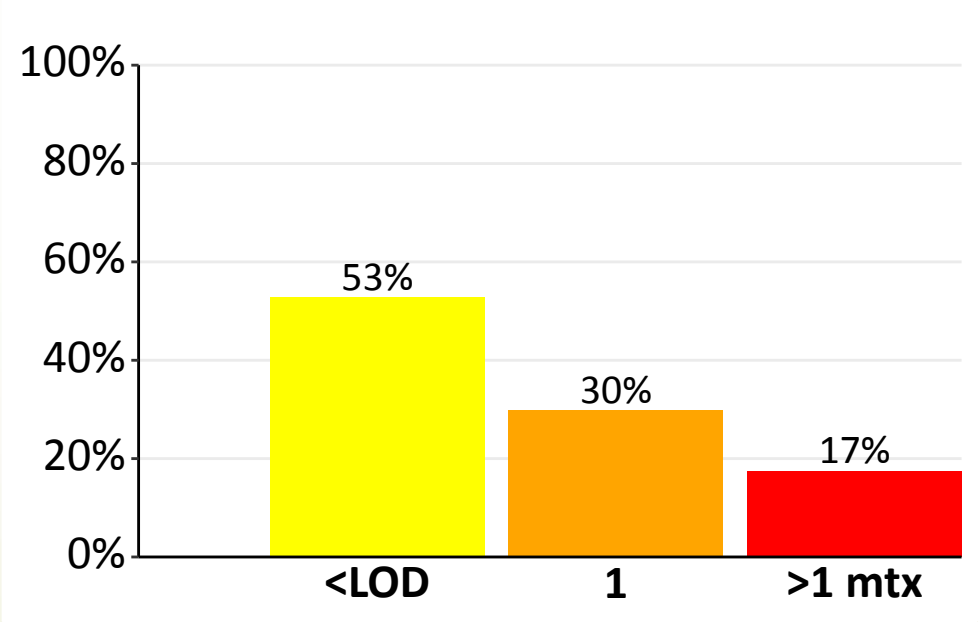
Hay and Grass Jan 2018 to Sep 2018

Total Risk Level: 24%*	Afla	ZEN	DON	T2	FUM	OTA
Number of samples tested	234	235	233	230	232	232
% Contaminated samples	2%	26%	12%	4%	25%	1%
% Above risk threshold	0%	19%	4%	2%	0%	0%
Average of positive (ppb)	21	1125	159	81	92	3
Median of positive (ppb)	1	215	130	77	44	2
Maximum (ppb)	79	10790	1015	189	327	4

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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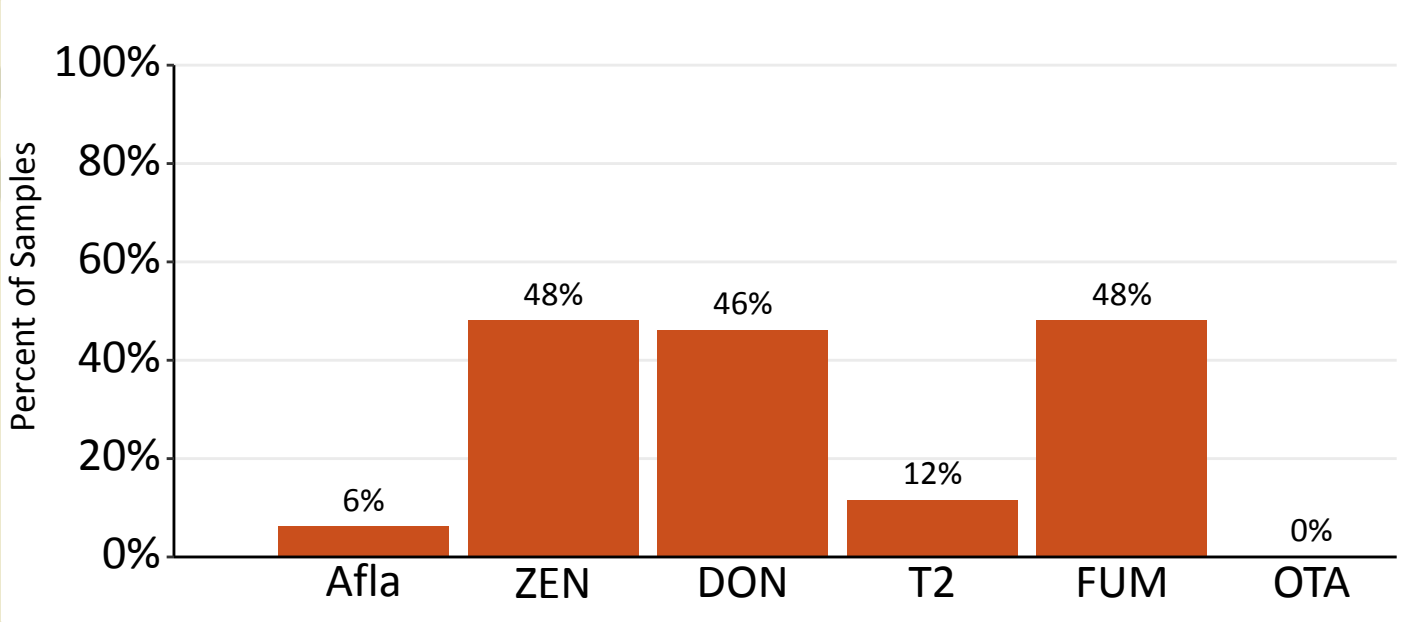
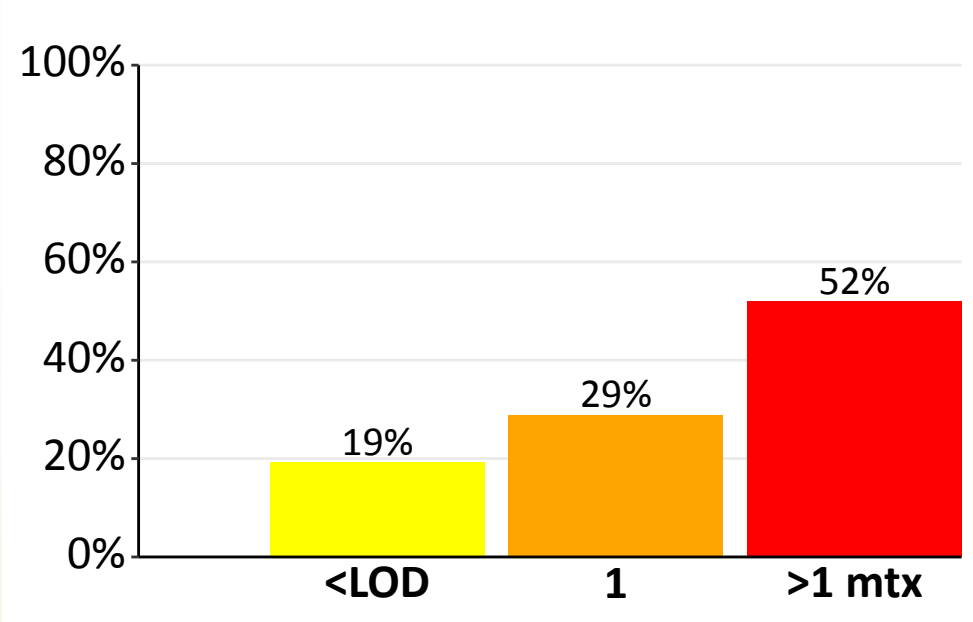
Straw Jan 2018 to Sep 2018

Total Risk Level: 25%*	Afla	ZEN	DON	T2	FUM	OTA
Number of samples tested	49	52	52	52	50	50
% Contaminated samples	6%	48%	46%	12%	48%	0%
% Above risk threshold	2%	10%	17%	2%	0%	0%
Average of positive (ppb)	4	375	162	20	61	-
Median of positive (ppb)	1	30	78	15	50	-
Maximum (ppb)	10	8641	760	66	184	-

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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Trouw : Aflatoxin Analysis – 2018/2019

- Overall result: 89 samples, average 31ppb, highest 150ppb, lowest 0ppb
- Maize: 22 samples, average 33ppb, highest 150ppb, lowest 1ppb
- Cattle feed: 7 samples, average 37ppb, highest 123ppb, lowest 18ppb

trouw nutrition
a Nutreco company

Aflatoxin B1 Levels found in Feed Mill, selectively buying for AFB1

		Number of Sample showing Aflatoxin B1 Levels (ppb)					
	Total Samples	**Blow 4**	**5 to 10**	**10 to 20**	**20 to 40**	**40 to 75**	**Above 75**
Maize	255	181	25	8	14	15	12
Rice Bran Ext. (DORB)	138	4	23	32	50	20	11
Cottonseed Ext	13	2	4	6	0	1	0
Whole Cotton Seeds	14	6	3	1	1	0	3
Cottonseed Cake	1	0	0	0	1	0	0
Rice Bran / polish	2	1	0	0	1	0	0
	423	**194**	**55**	**47**	**67**	**36**	**26**

Aflatoxin B1 Levels found in Feed Mill, selectively buying for AFB1

Percentage of Samples Above 20 ppb

Ingredient	% Samples
Maize	16
Rice Bran Ext. (DORB)	59
Cottonseed Ext	8
Whole Cotton Seeds	29
Rice Bran / polish	50

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Feed Production

- Conditioning
- Cooling

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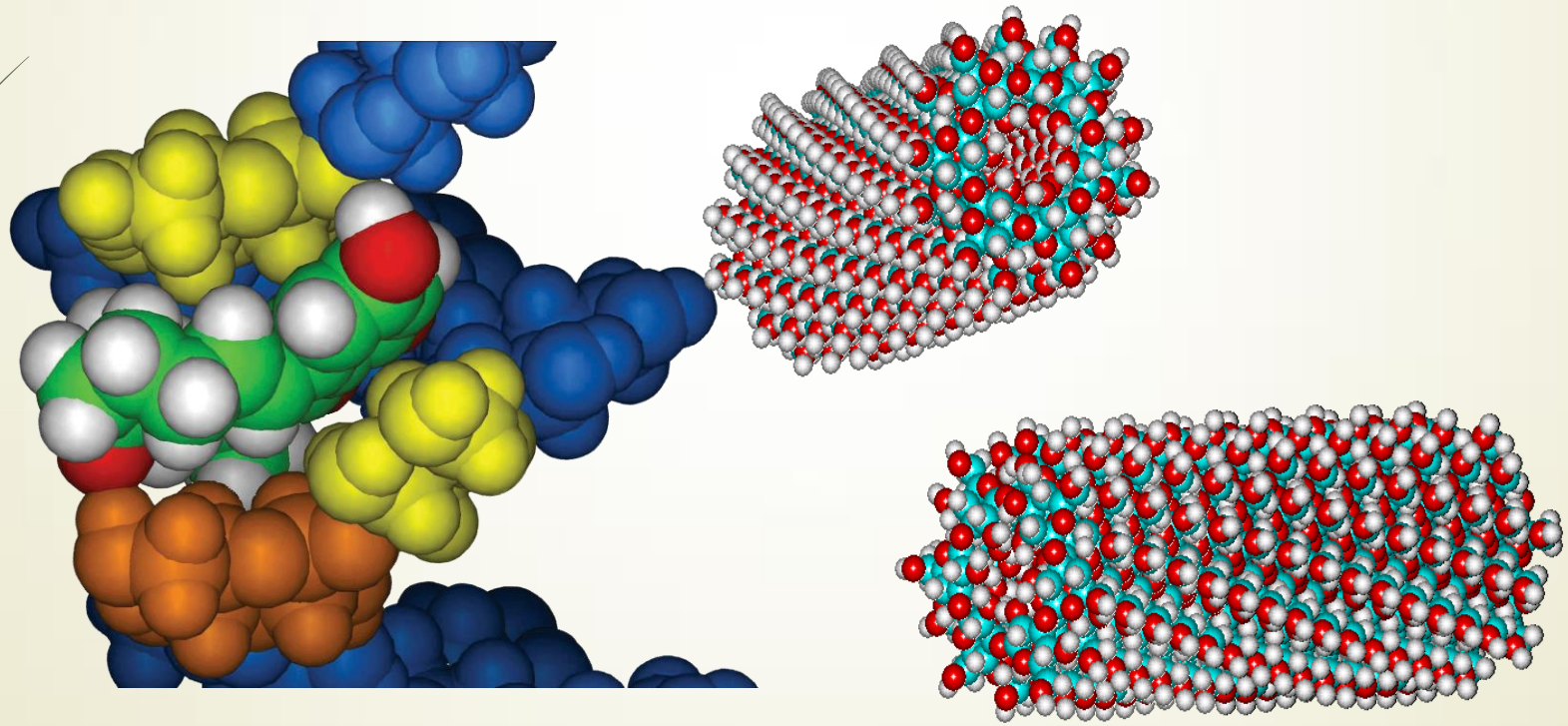
Feed Storage

Feed Additives

- Antifungals? Can cause increased production of mycotoxins
- Sequestering agents



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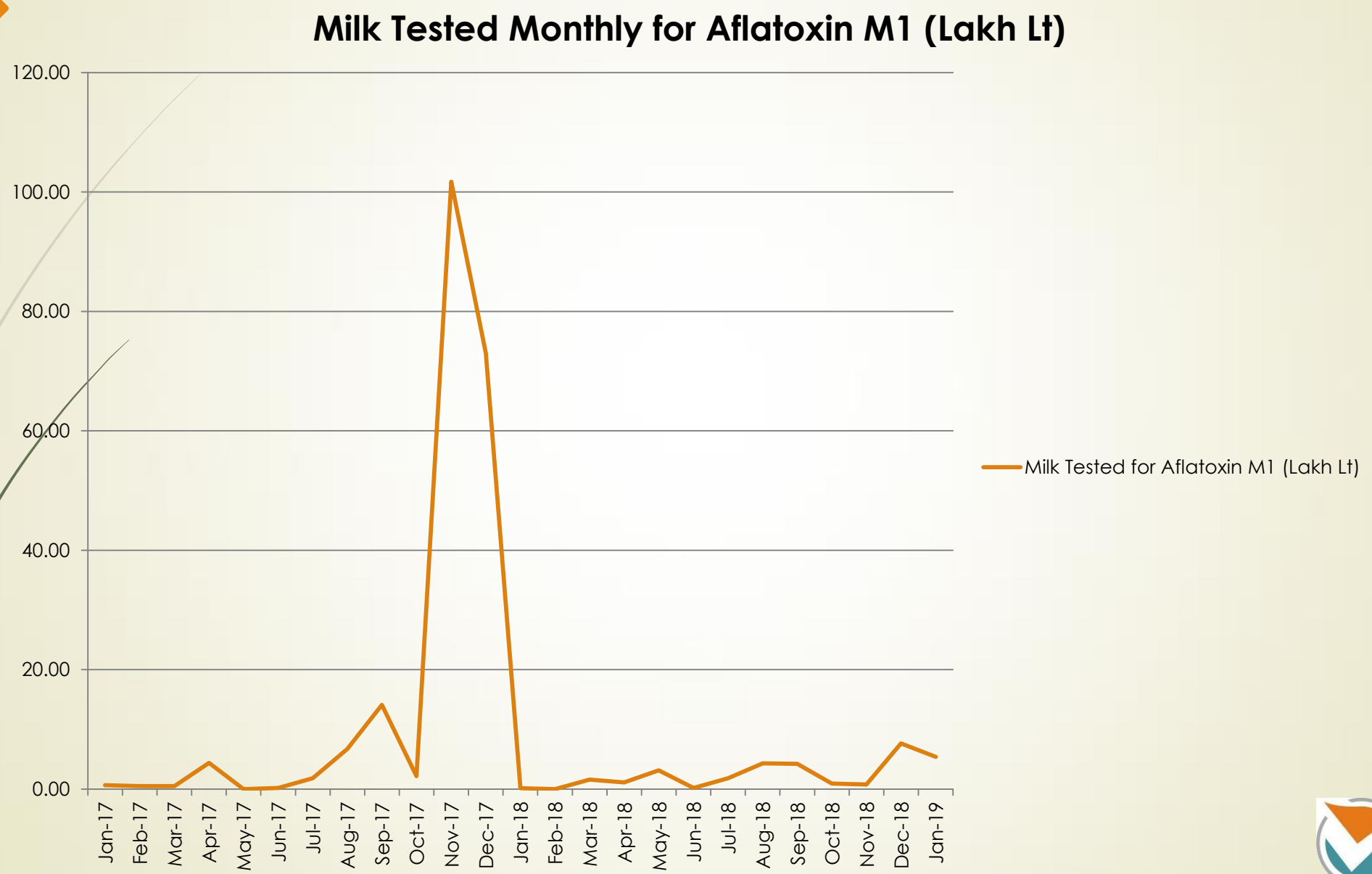
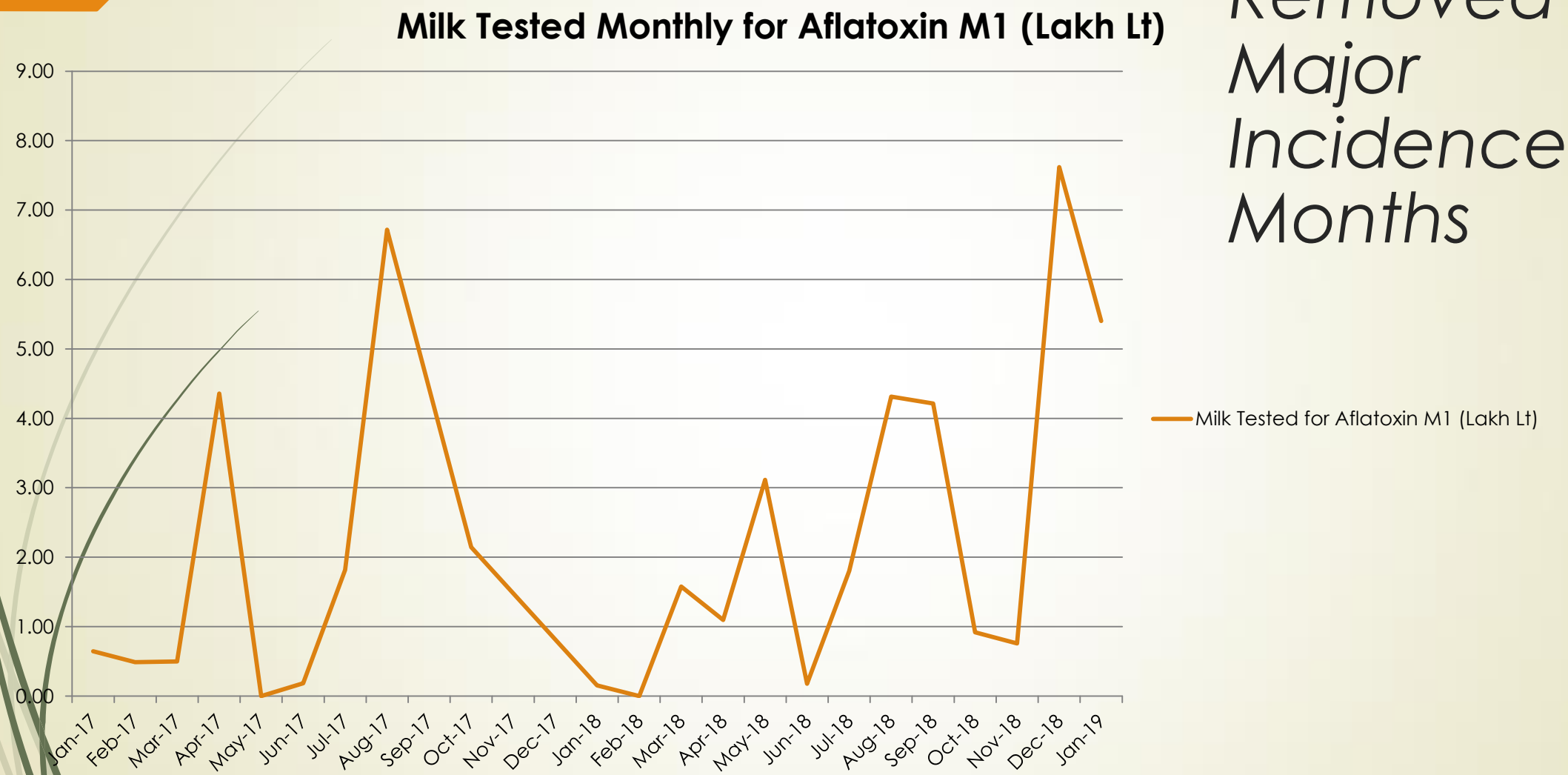
AFM1 Incidences



AFM1 Incidences



Removed Major Incidence Months

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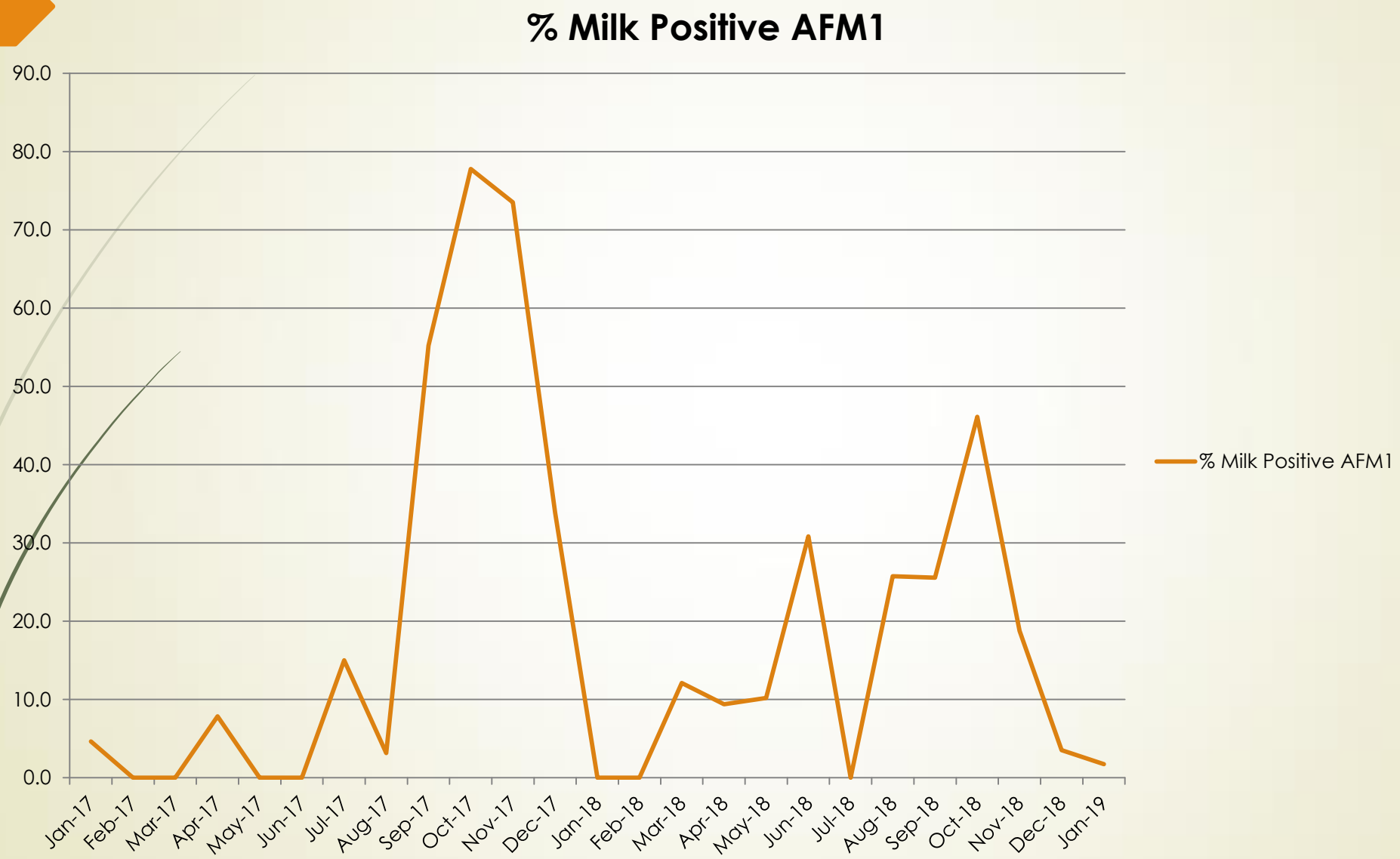
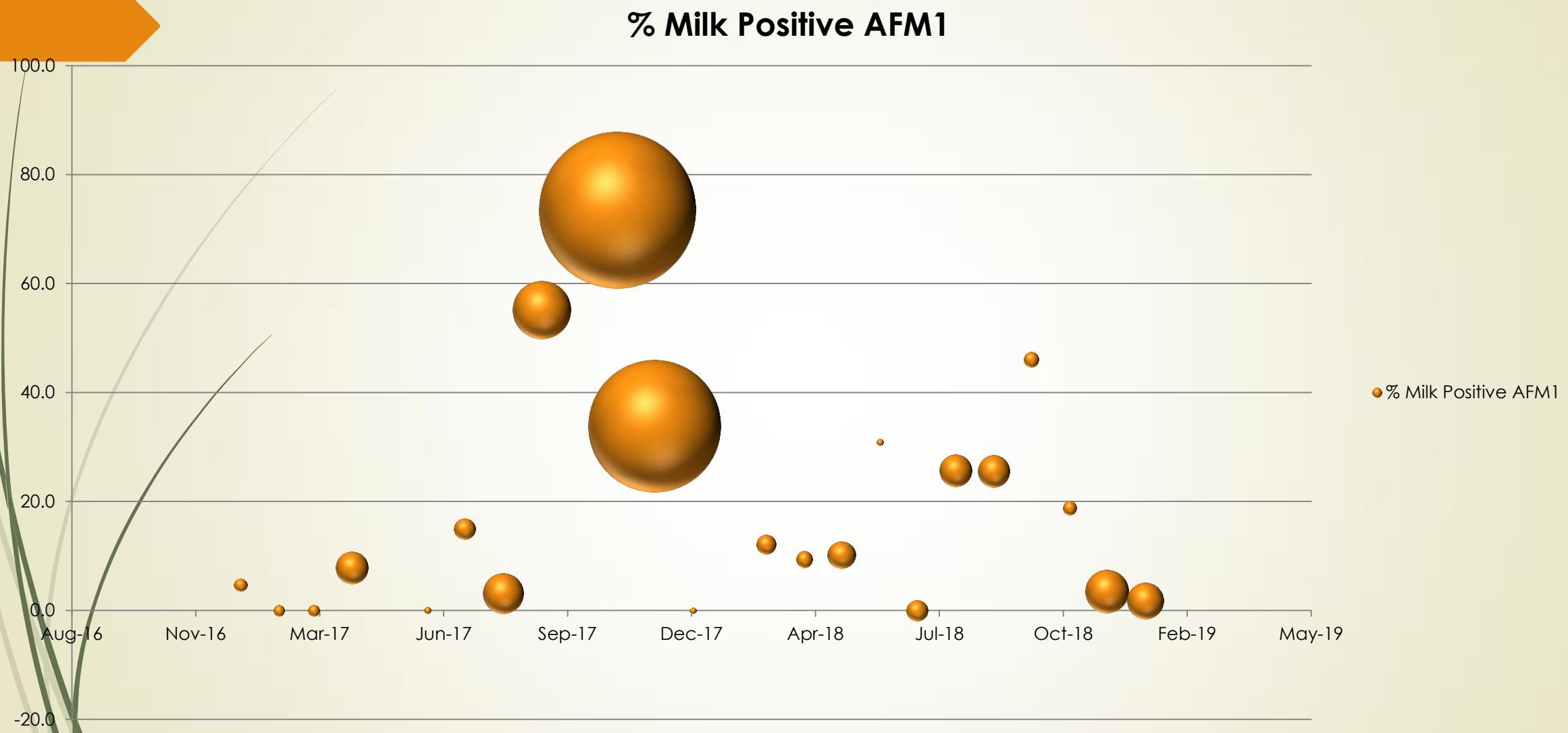
AFM1 Incidences



AFM1 Incidences



VIZIVA SERVICES

Complex Problem

VIZIVA SERVICES

Sampling

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20 ppb

- One maize grain weigh roughly 0.3 gm
- If 1 tuck is 16 MT, then
- 1 maize grain in 19 trucks = 1 ppb
- 20 maize grains in 19 trucks = 20 ppb

Sampling :
Fungus occurs in pockets!

Moisture Analysis

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Moisture Analysis

- Temperature
- Time
- Surface Area
- Moisture re-absorption

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Moisture Analysis

	BIS	AOAC	GAFTA
Sample & Container size	?	?	✓
Temperature	100 ^{0}C	135 ^{0}C	103 ^{0}C
Time	2 hours repeated	2 hours	4 hours
Moisture re-absorption / Desiccator	?	?	?

Difference of 0.5% - enough to take feed from "safe" to "prone to fungal growth"

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Analysis of Aflatoxins in feed and Ingredients

- Approved methods ..... ?

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Analysis of Aflatoxins in feed and Ingredients Approved Methods ....?

- The Grain Inspection, Packers and Stockyards Administration - USDA

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FGIS Performance Verified Aflatoxin Test Kits – Effective 12/19/2018

Manufacturer	Test Kit	Part Number	FOL Code	Test Type and Test Kit Range	Approved Commodities are Listed Below. Equivalent Commodities can be Found in the Test Kit Design Criteria Appendix C. (page No. 14-15)	Detection Method	Certificate Expiration Date	Official Instructions
Charm Sciences, Inc.	ROSA FAST Aflatoxin Quantitative Test	LF-AFQ-FAST	AFLRA	Aflatoxin 5 – 300 ppb	**Organic Solvent Extraction**: corn, barley, brown rice, corn germ meal, corn gluten meal, corn/soy blend, distillers dried grain (DDG), distillers dried grain with solubles (DDGS), hominy, millet, oats, popcorn, rough rice, rye, sorghum, soybean hulls, soybean meal, soybeans, wheat	EZ-M Reader (LF-ROSA-EZ-M) REQUIRES CALIBRATION UPDATE FOR USE	3/06/2021	LF-AFQ-FAST Effective 02-23-2018
Charm Sciences, Inc.	ROSA WET-S5 Aflatoxin Quantitative Test	LF-AFQ-WETS5	AFLRF	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn, distillers dried grain with solubles (DDGS), sorghum, soybeans, and wheat	EZ-M Reader (LF-ROSA-EZ-M)	6/14/2020	LF-AFQ-WETS5 Effective 06-14-2017
Charm Sciences, Inc.	ROSA WET-S3 Aflatoxin Quantitative Test	LF-AFQ-WETS3	AFLRE	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn	ROSA-M Reader (LF-ROSAREAD-ER-M-NB) EZ-M Reader (LF-ROSA-EZ-M)	8/16/2019	LF-AFQ-WETS3 Effective 08-05-2016
EnviroLogix, Inc.	QuickTox Kit for Quick-Scan Aflatoxin Flex	AQ 309 BG	AFLJC	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn , sorghum **Organic Solvent Extraction**: distillers dried grains with solubles (DDGS)	EnviroLogix QuickScan System	11/16/2019	AQ-309-BG Effective 05-07-2018
Neogen Corporation	Reveal Q+ MAX for Aflatoxin	8088	AFLGF	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn, flaking corn grits, pearled barley, popcorn, brown rice, milled rice, sorghum, soybean, and wheat	AccuScan Gold Reader AccuScan Pro Reader	7/29/2019	8088 Effective 05-01-2018
Neogen Corporation	Reveal Q+ for Aflatoxin	8085	AFLGC	Aflatoxin 5 – 300 ppb	**Organic Solvent Extraction**: corn, flaking corn grits, corn germ meal, corn gluten meal, corn/soy blend, corn starch, distillers dried grains with solubles (DDGS), milled rice, popcorn, rough rice, sorghum, and wheat	AccuScan Gold Reader	5/31/2021	8085 Effective 05-29-2018
Neogen Corporation	Reveal Q+ for Aflatoxin	8085	AFLGC	Aflatoxin 5 – 300 ppb	**Organic Solvent Extraction**: Corn, corn germ meal, corn gluten meal, corn/soy blend, corn starch, distillers dried grain with solubles (DDGS), popcorn, rough rice, sorghum, and wheat.	Raptor Integrated Analysis Platform	12/12/2021	8085 Effective 12-12-2018
Neogen Corporation	Veratox Aflatoxin Quantitative Test	8030	AFLGB	Aflatoxin 5 – 300 ppb	**Organic Solvent Extraction**: corn, corn germ meal, corn gluten meal, corn/soy blend, distillers dried grains with solubles (DDGS), malted barley, brown rice, milled rice, millet, pearl or pearled barley, popcorn, rice bran, rye, sorghum, soybeans, white rye flour, wheat, and wheat bran.	Stat Fax Reader Model 321 Plus and Model 4700	4/12/2021	8030 Effective 04-11-2018
Neogen Corporation	Veratox Aflatoxin Quantitative Test	8035	AFLGE	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn, pearled barley, corn gluten meal, distiller dried grains with solubles, popcorn, rye, sorghum, soybeans, and wheat	Stat Fax Reader Model 321 Plus and Model 4700	8/30/2019	8035 Effective 08-29-2016
R-Biopharm, Inc.	RIDA SCREEN FAST Aflatoxin ECO	R5201	AFLGG	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn, corn gluten meal, corn/soy blend, corn starch, malted barley, milled rice, popcorn, sorghum, soybeans, and wheat	Stat Fax Reader Model 303 Plus	4/24/2020	R5201 Effective 11-20-2017
Romer Labs, Inc.	FluoroQuant Afla	COKFA1010	AFLF	Aflatoxin 5 – 300 ppb	**Organic Solvent Extraction**: corn, corn/soy blend, milled rice, brown rice, popcorn, rough rice, sorghum, wheat	Romer FQ-Reader Model EQFFM3000	4/4/2021	COKFA1010 Effective 03-21-2018
Romer Labs, Inc.	AgraStrip Total Aflatoxin Quantitative Test WATEX	COKAS1600W	AFLLA	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn	Romer Agra Vision Reader Model EQASR1000	8/18/2020	COKAS1600W Effective 08-09-2017

VIZIVA SERVICES

FGIS Performance Verified Aflatoxin Test Kits – Effective 12/19/2018

Manufacturer	Test Kit	Part Number	FOL Code	Test Type and Test Kit Range	Approved Commodities are Listed Below. Equivalent Commodities can be Found in the Test Kit Design Criteria Appendix C. (page No. 14-15)	Detection Method	Certificate Expiration Date	Official Instructions
Charm Sciences, Inc.	ROSA FAST Aflatoxin Quantitative Test	LF-AFQ-FAST	AF		**Organic Solvent Extraction**: corn, barley, brown rice, corn germ meal, corn gluten meal, corn/soy blend, distill-ers dried grain (DDG), distillers dried grain with solubles (DDGS), hominy, millet, oats, popcorn, rough rice, rye,	Reader SA-EZ-M) UIRES RATION FOR USE	3/06/2021	LF-AFQ-FAST Effective 02-23-2018
Neogen Corporation	Veratox Aflatoxin Quantitative Test	8035	AFLGE	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn, pearled barley, corn gluten meal, distiller dried grains with solubles, popcorn, rye, sorghum, soybeans, and wheat	Stat Fax Reader Model 321 Plus and Model 4700	8/30/2019	8035 Effective 08-29-2016
R-Biopharm, Inc.	RIDA SCREEN FAST Aflatoxin ECO	R5201	AFLGG	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn, corn gluten meal, corn/ soy blend, corn starch, malted barley, milled rice, pop-corn, sorghum, soybeans, and wheat	Stat Fax Reader Model 303 Plus	4/24/2020	R5201 Effective 11-20-2017
Romer Labs, Inc.	FluoroQuant Afla	COKFA1010	AFLF	Aflatoxin 5 – 300 ppb	**Organic Solvent Extraction**: corn, corn/soy blend, milled rice, brown rice, popcorn, rough rice, sorghum, wheat	Romer FQ-Reader Model EQFFM3000	4/4/2021	COKFA1010 Effective 03-21-2018
Romer Labs, Inc.	AgraStrip Total Aflatoxin Quantitative Test WATEX	COKAS1600W	AFLLA	Aflatoxin 5 – 300 ppb	**Water-Based Extraction**: corn	Romer Agra Vision Reader Model EQASR1000	8/18/2020	COKAS1600W Effective 08-09-2017

Organic Solvent Extraction: corn, barley, brown rice, corn germ meal, corn gluten meal, corn/soy blend, distill-ers dried grain (DDG), distillers dried grain with solubles (DDGS), hominy, millet, oats, popcorn, rough rice, rye, sorghum, soybean hulls, soybean meal, soybeans, wheat

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Aflatoxin Levels in Feed & Excretion in Milk

Levels in Feed & Excretion in Milk

- Generally assumed to be 1.5 to 3%
- Varies highly
- Several factors :
  - Rumen health
  - Liver function (Hepatic Biotransformation Capacity)
  - Udder health (integrity of the mammary alveolar cell membranes)
  - **Most importantly – Milk Yield & Stage of Lactation**

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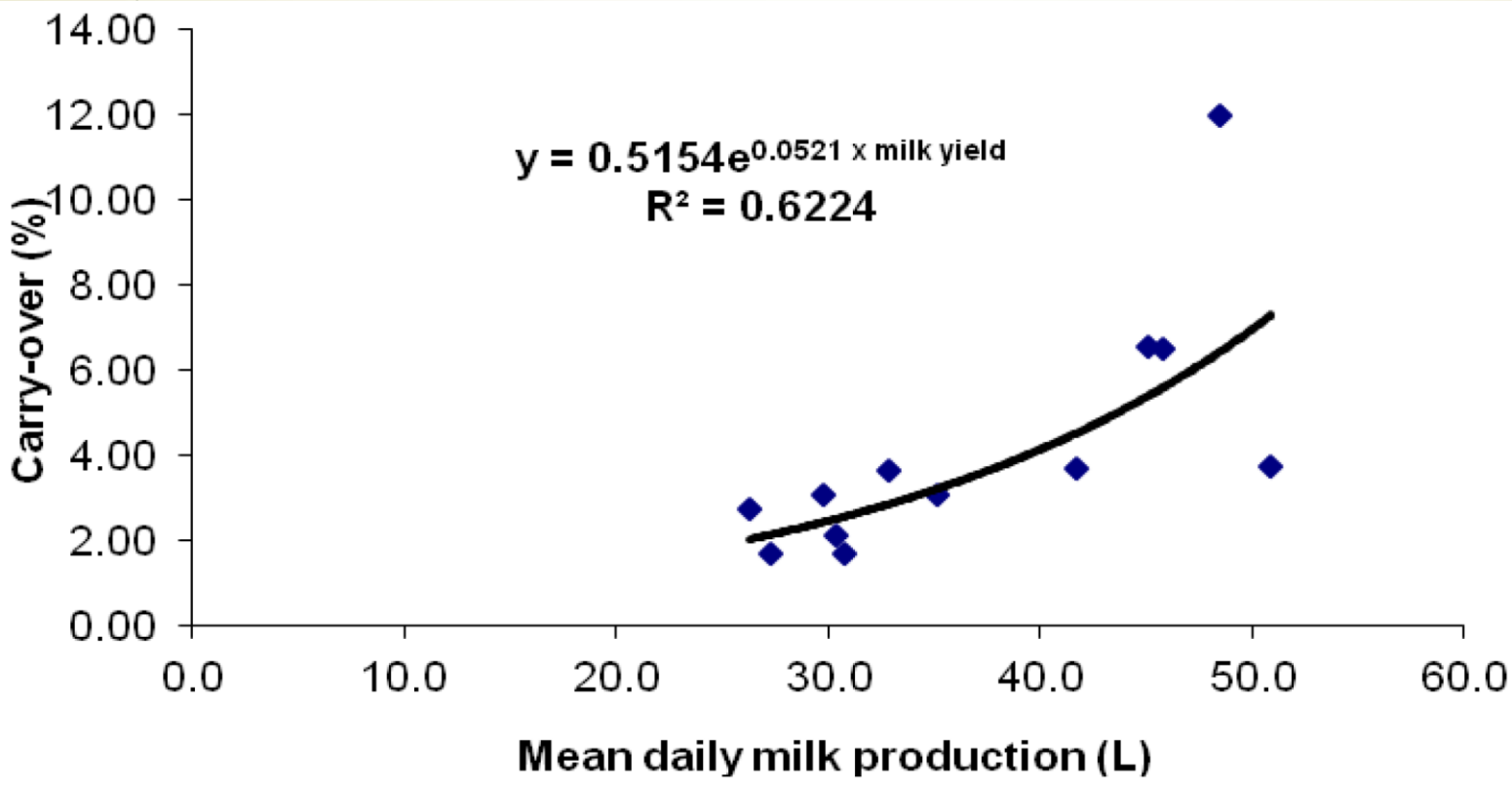
Aflatoxin Excretion in Milk - % of Ingestion



Source : Malka *et al* Carry-Over of Aflatoxin B1 to Aflatoxin M1 in High Yielding Israeli Cows in Mid- and Late-LactationToxins 2013, 5(1), 173-183

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Factors Affecting Excretion

- Health of cow
- Forage

Sampling of forages

- Silage

Will rarely show high levels of aflatoxin

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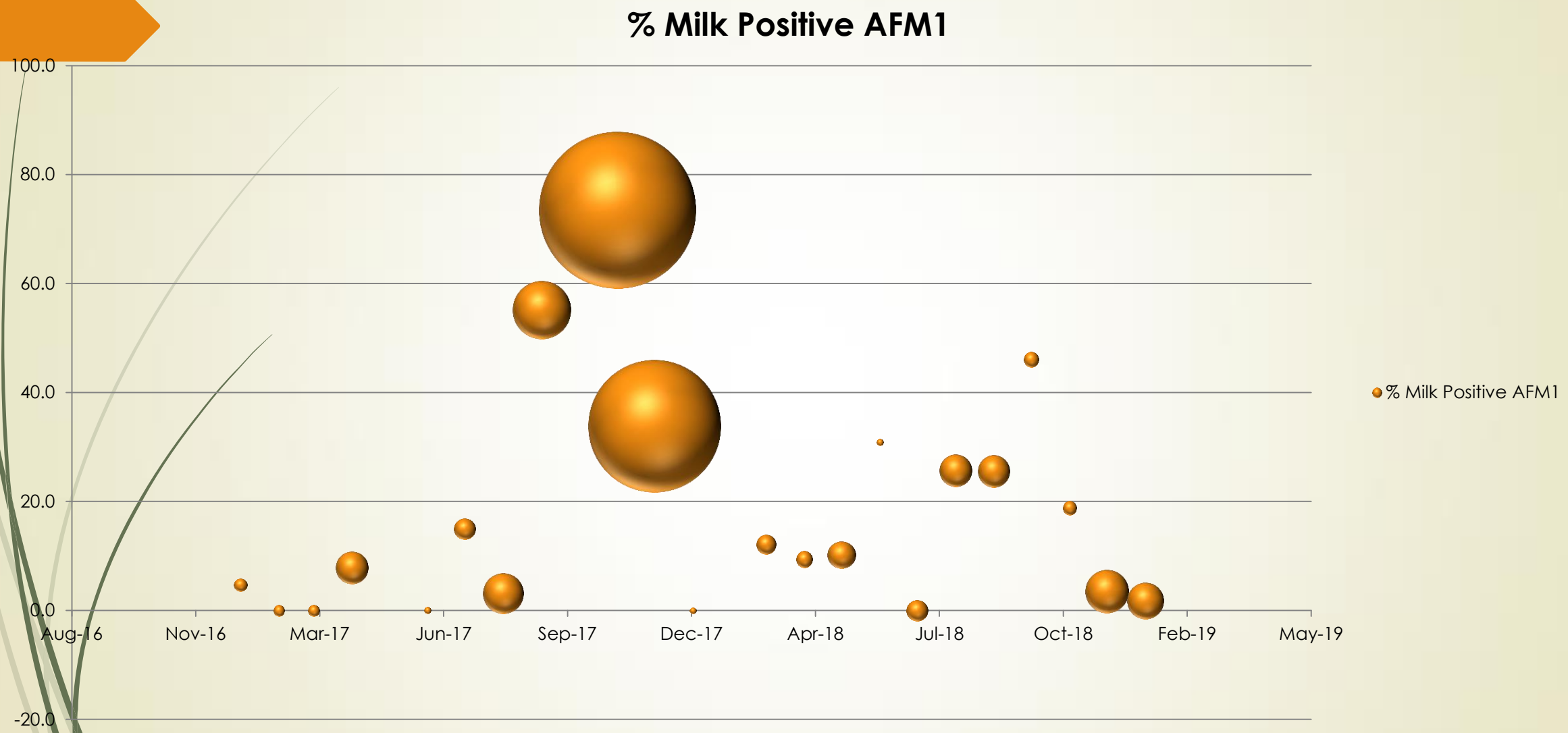
Factors Affecting Excretion

- Forage

AFM1 Incidences



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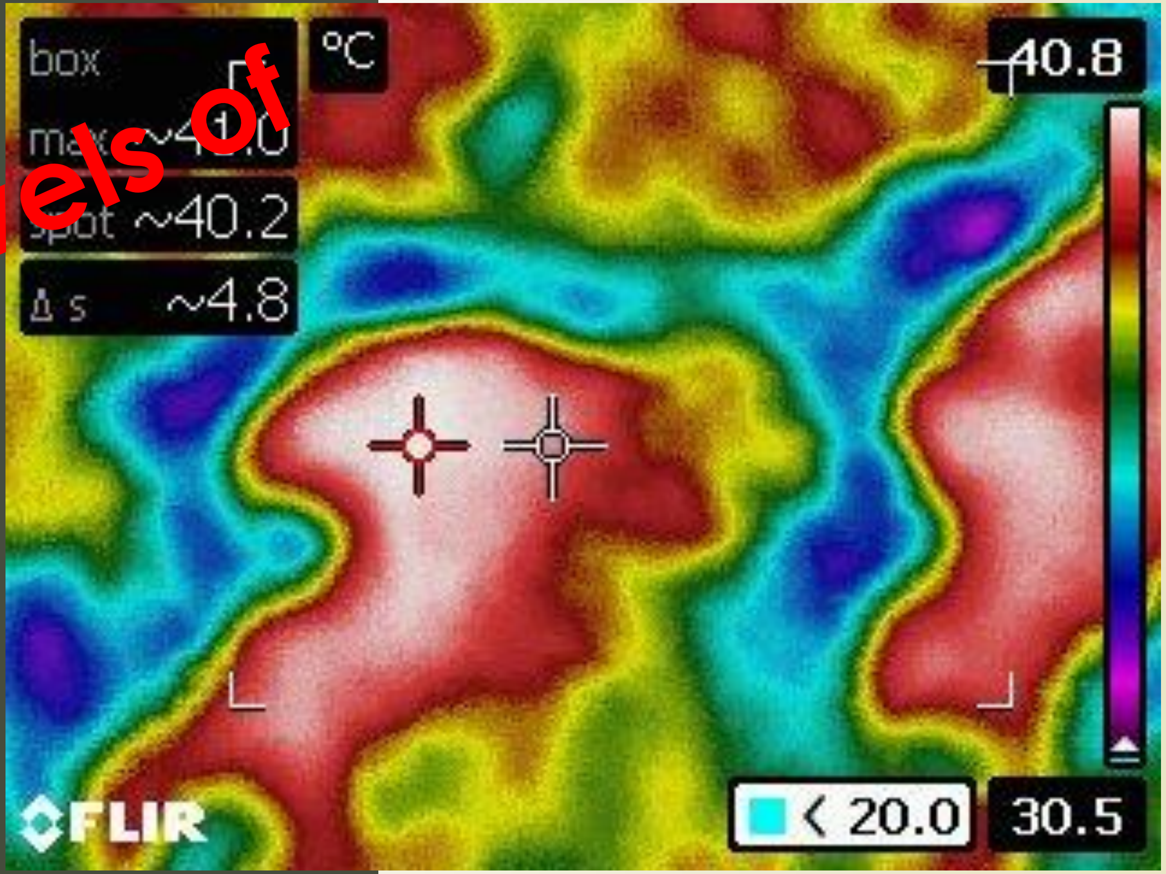
Green chops

VIVA SERVICES

Green chops



Will rarely show high levels of aflatoxin

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Wet Byproducts

- Distiller's Wet Grains
- Maize fibre
- Wet Maize gluten feed
- Tapioca Thippy

Will rarely show high levels of aflatoxin

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Some Unsolicited Advice

For Dr. Shreedhar

Aflatoxins Testing

- Standardization
- Developing Indigenous vendors for ELISA kits
- Offer finanial assistance for testing
- Collate data on large scale

Cost of Investigation

Thank You