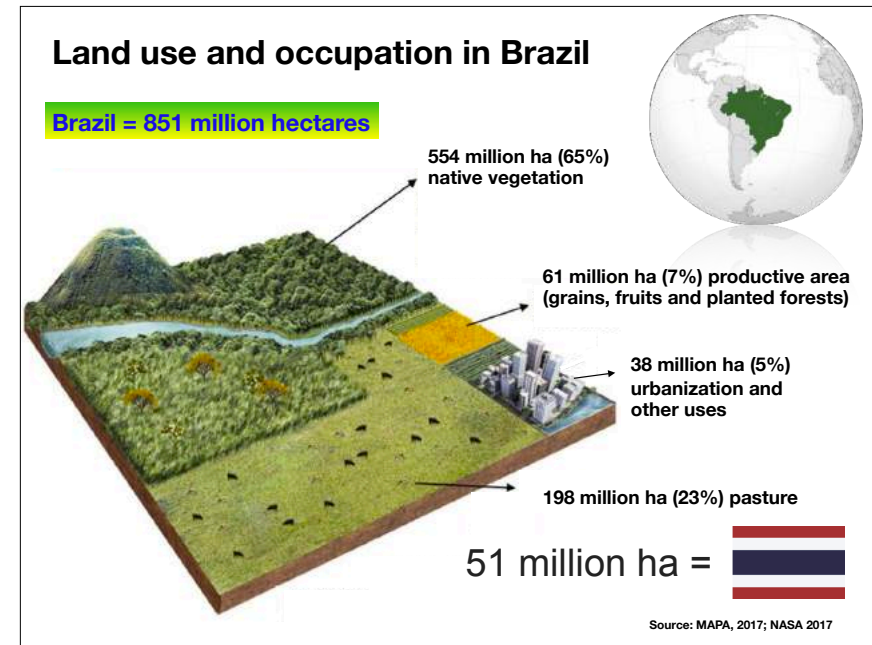
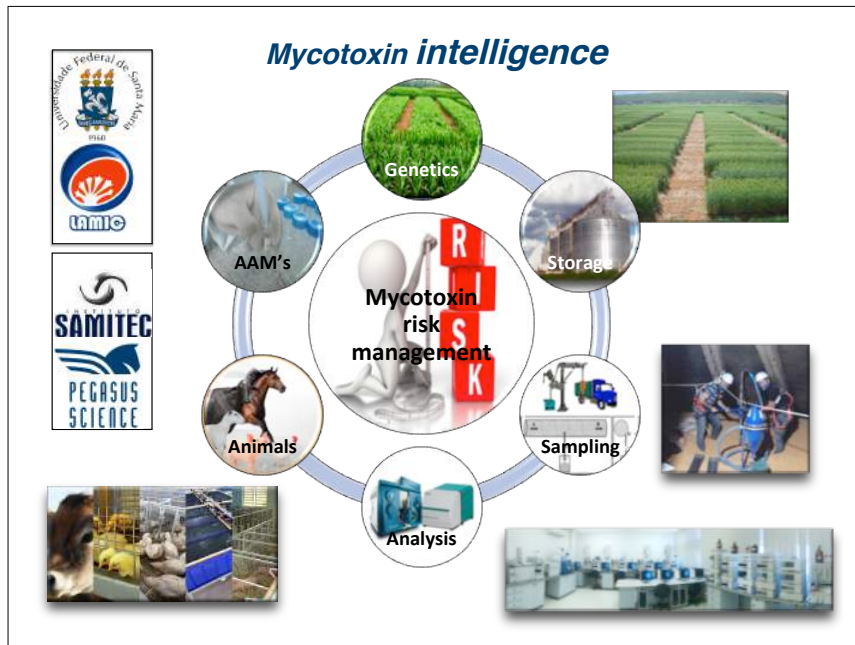




Mycotoxins, diagnose and detoxification strategies

- ✓ **BACKGROUND AND APPROACH**
- ✓ **MYCOTOXINS ON ANIMAL HEALTH**
 - **Most important mycotoxins**
 - **Effects on animals**
 - **Clinical**
 - **Economical**
- ✓ **"State of Art"**
- ✓ **WAYS TO EVALUATE AntiMycotoxins Additives (AMA's)**
 - *in vitro*
 - *in vivo*
 - **Results**





MYCOTOXINS ON ANIMAL HEALTH AND WAYS TO EVALUATE ANTIMYCOTOXIN ADDITIVES

- ✓ **BACKGROUND AND APPROACH**
- ✓ **MYCOTOXINS ON ANIMAL HEALTH**
 - Most important mycotoxins
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 - Clinical
 - Economical
- ✓ **LATEST NEWS**
- ✓ **WAYS TO EVALUATE AntiMycotoxins Additives (AMA's)**
 - *in vitro*
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 - Results

Most important mycotoxins in Brazil

Aflatoxins

Zearalenone

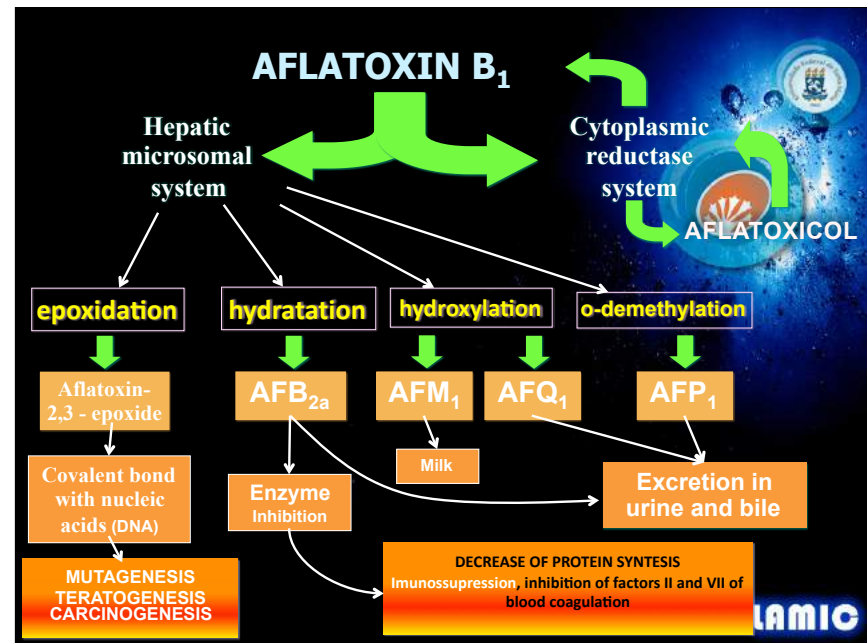
Fumonisin

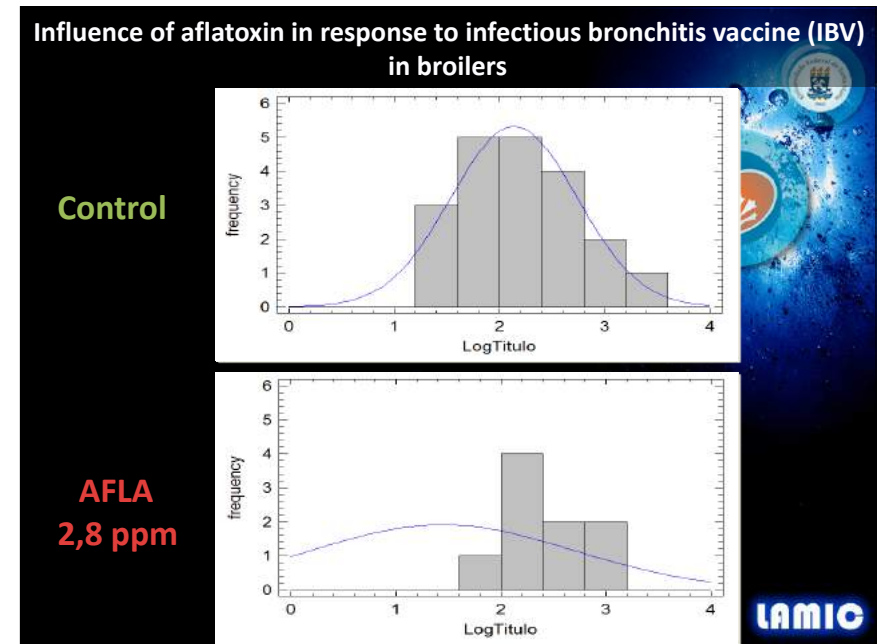
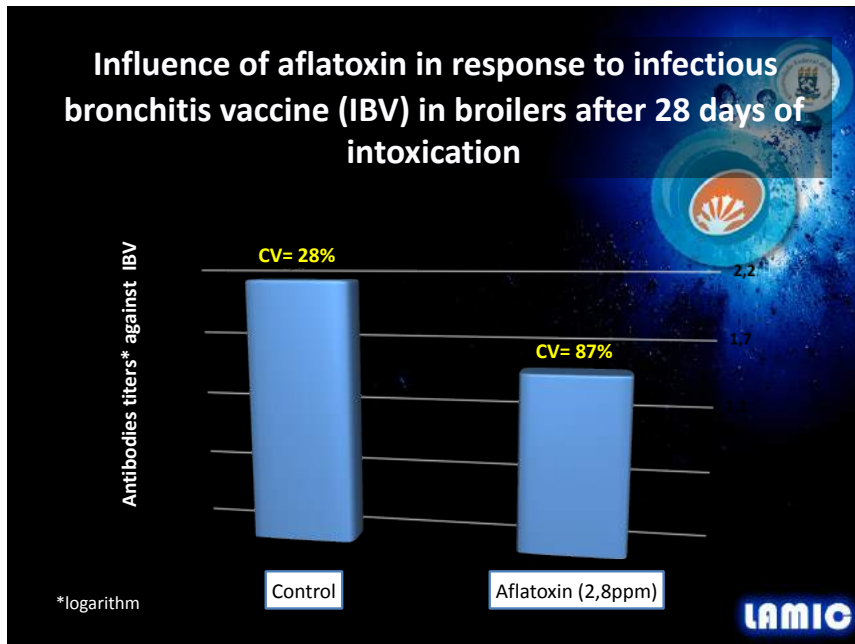
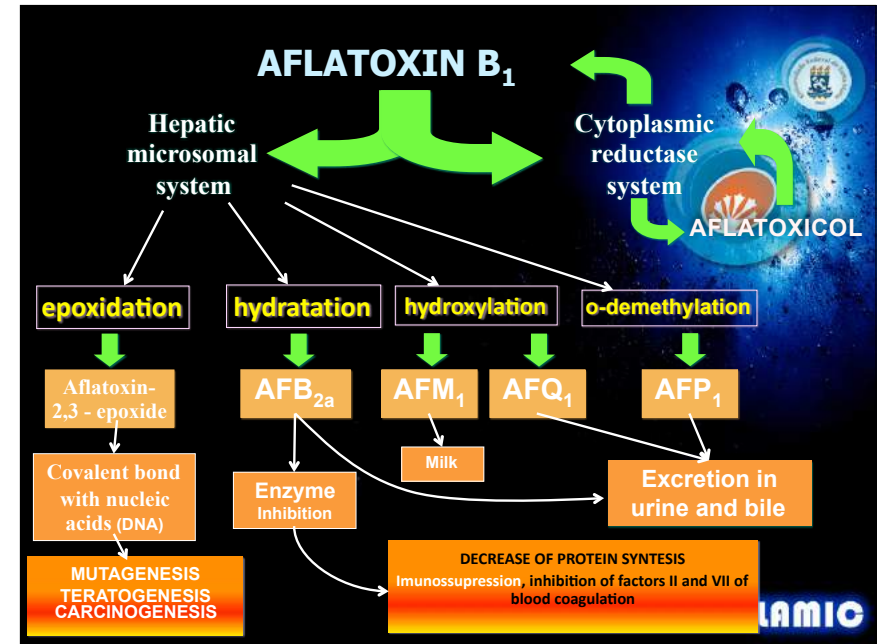
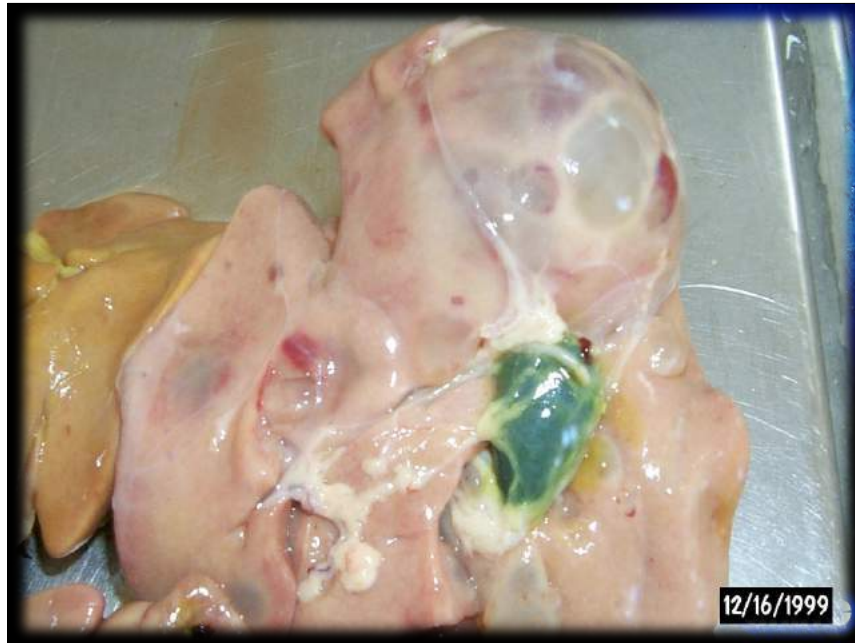
Trichothecenes: DON
T-2 Toxin
DAS...

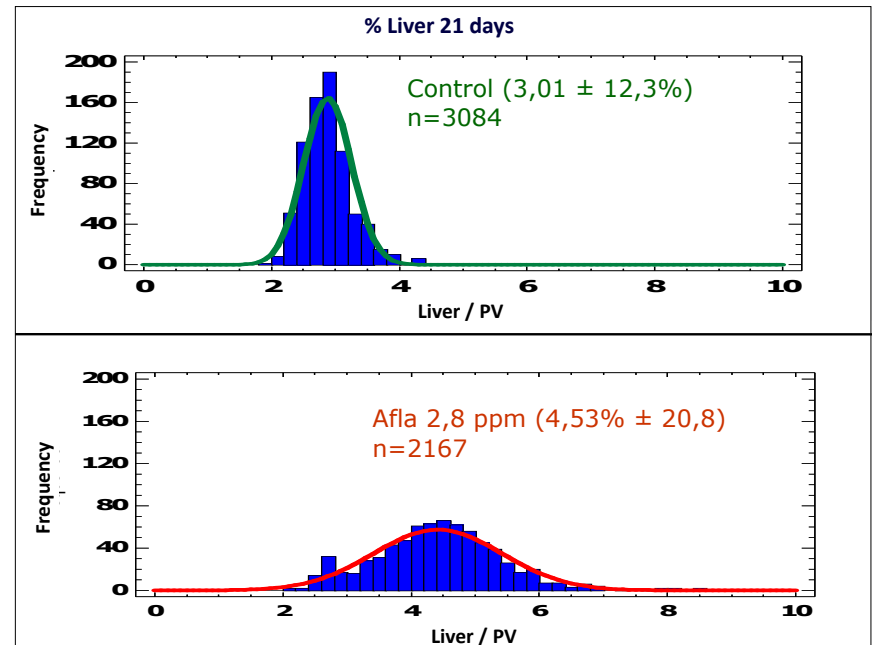
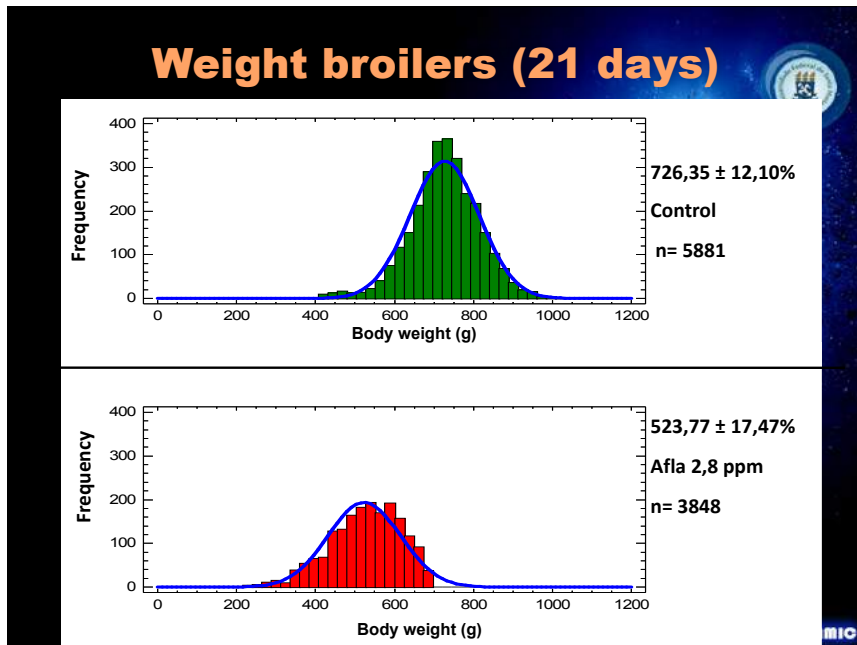
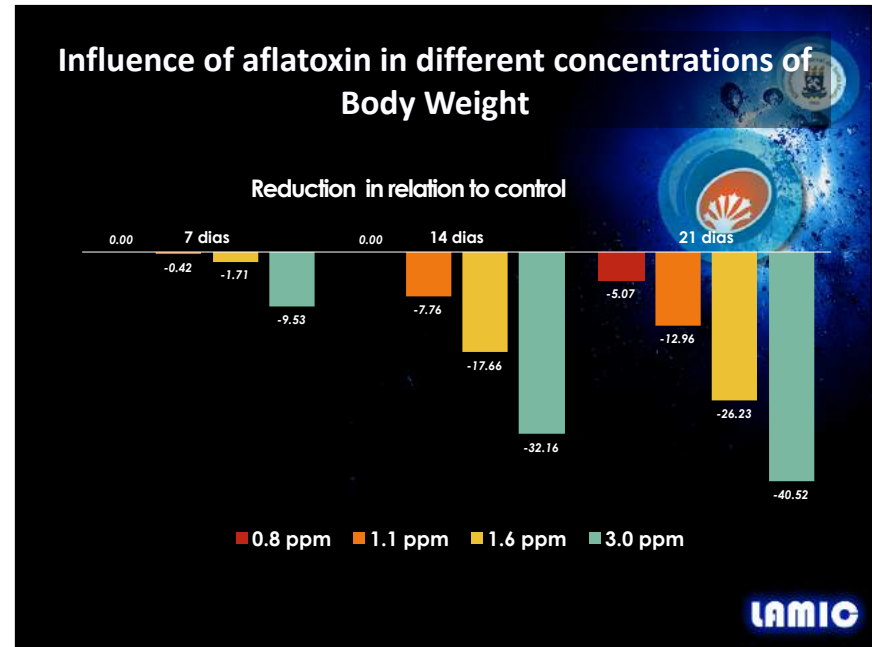
AFLATOXINS

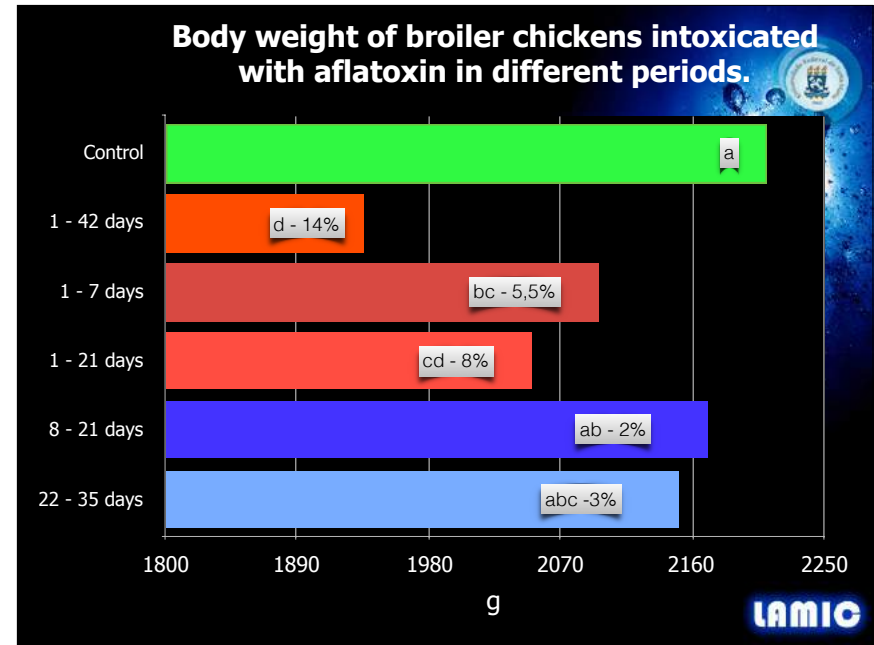
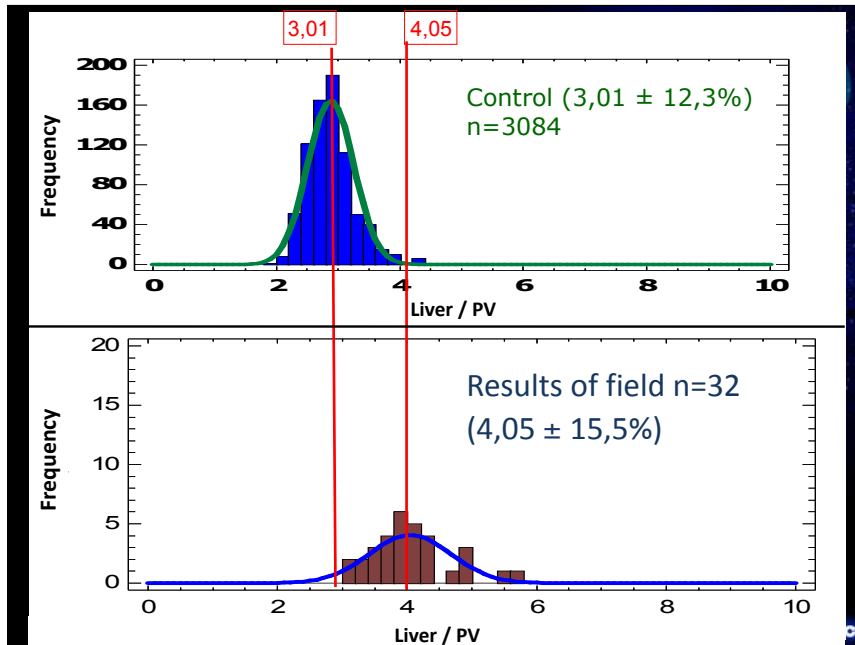
AFLATOXINS

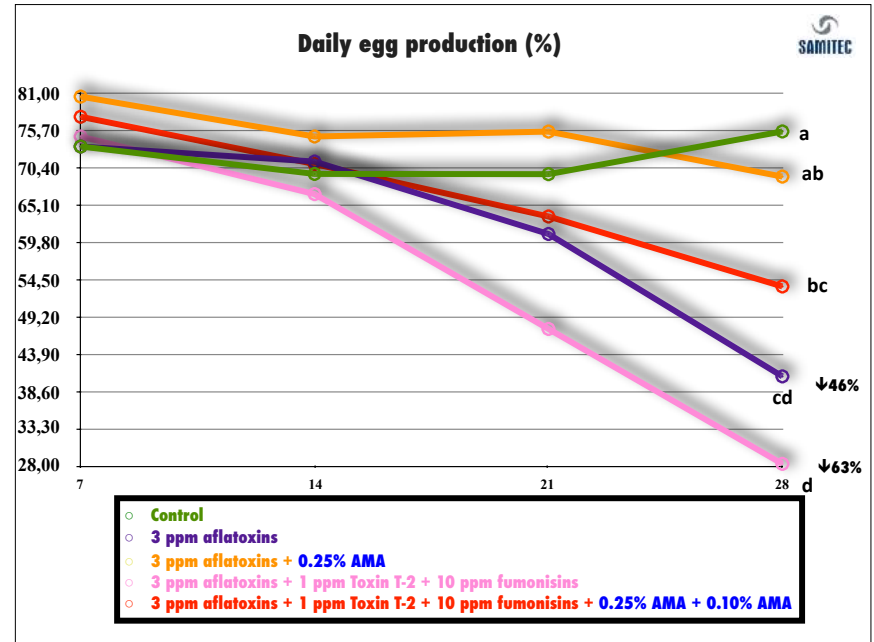
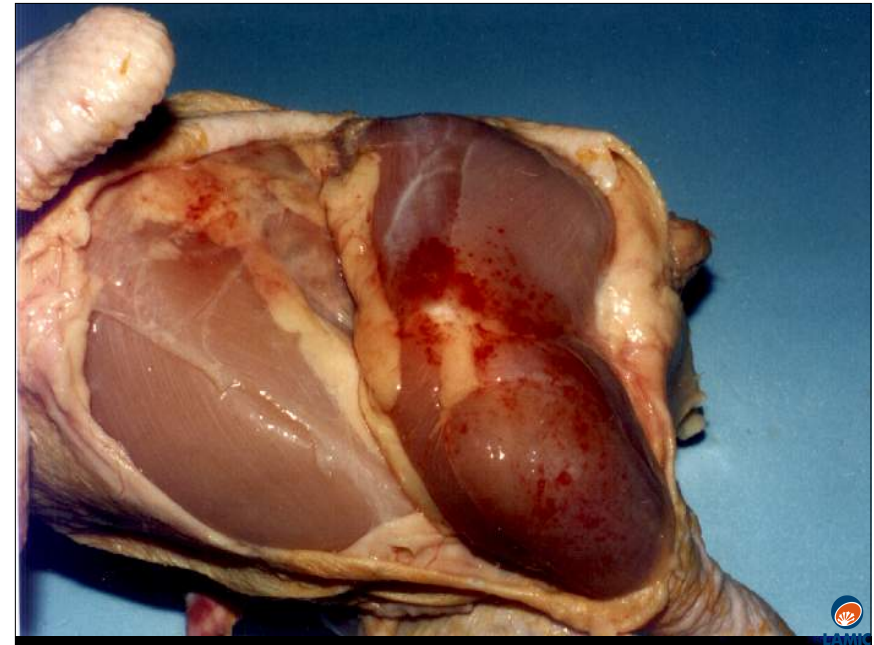
- Aspergillus flavus*
- A. parasiticus*
- A. nominus*
- A. pseudotamari*
- A. bombycis*














RESISTANCE GENETICS



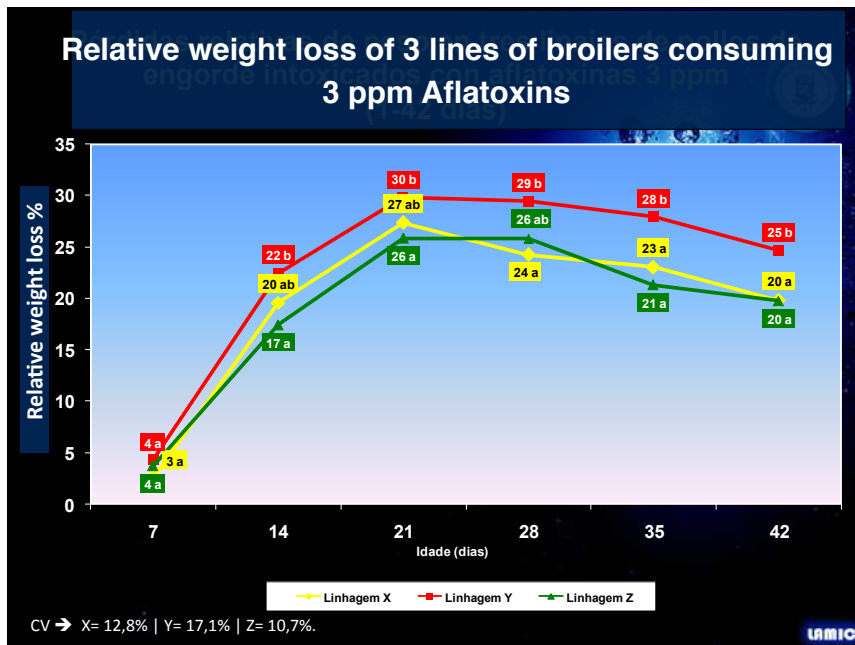
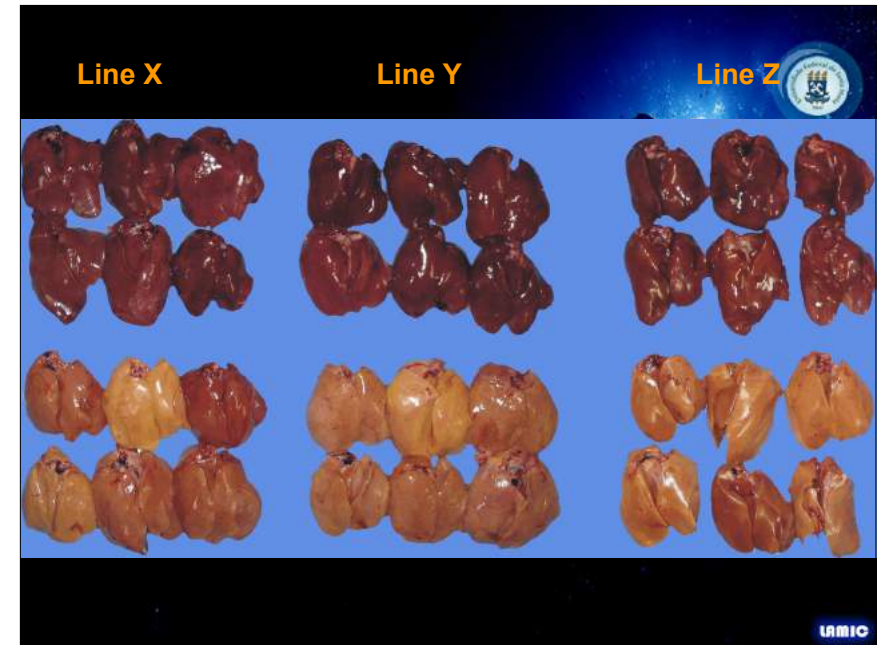
LAMIC
Resultado de Qualidade



U. F. P. A. - PA.
SANTA MARIA
BRASIL



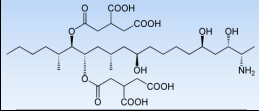

LAMIC PUBLICA NOVO ESTUDO SOBRE AFLATOXINAS
LAMIC estuda **impacto das aflatoxinas** no desempenho de três linhagens de frangos de corte.
>>> LEIA AQUI: www.lamic.ufsm.br



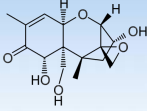


Most important Fusariotoxins

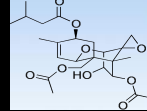
- Zearalenone
- Fumonisin
- T-2 toxina
- Deoxynivalenol (DON)
- Diacetoxycirpenol (DAS)
- Nivalenol



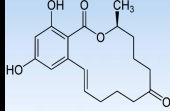
Fumonisin B₁



Desoxynivalenol



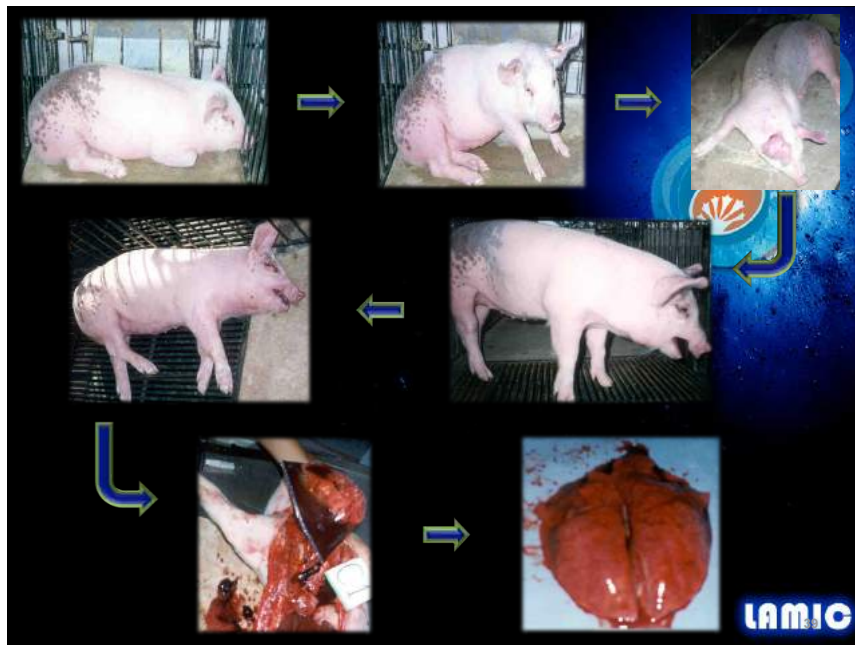
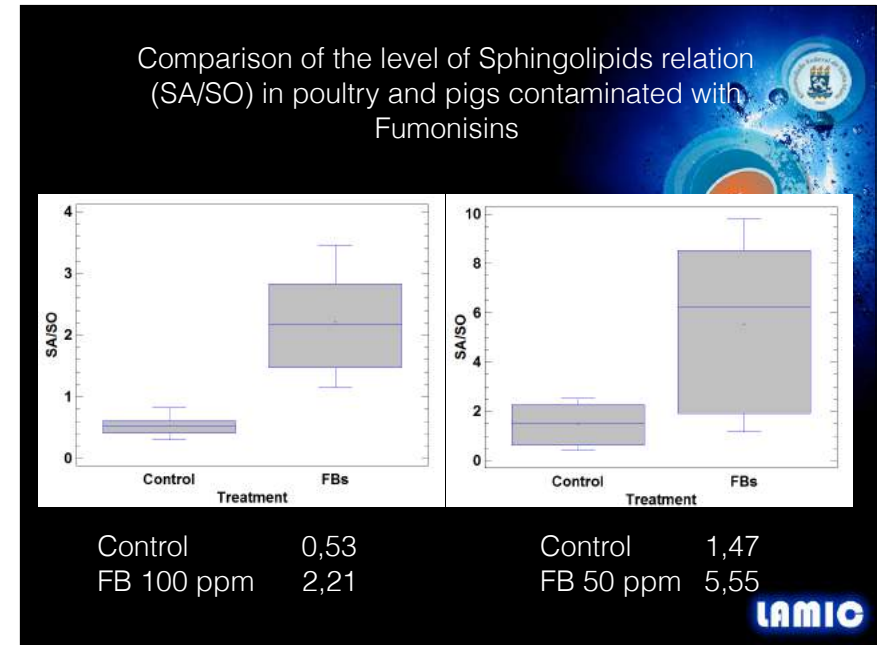
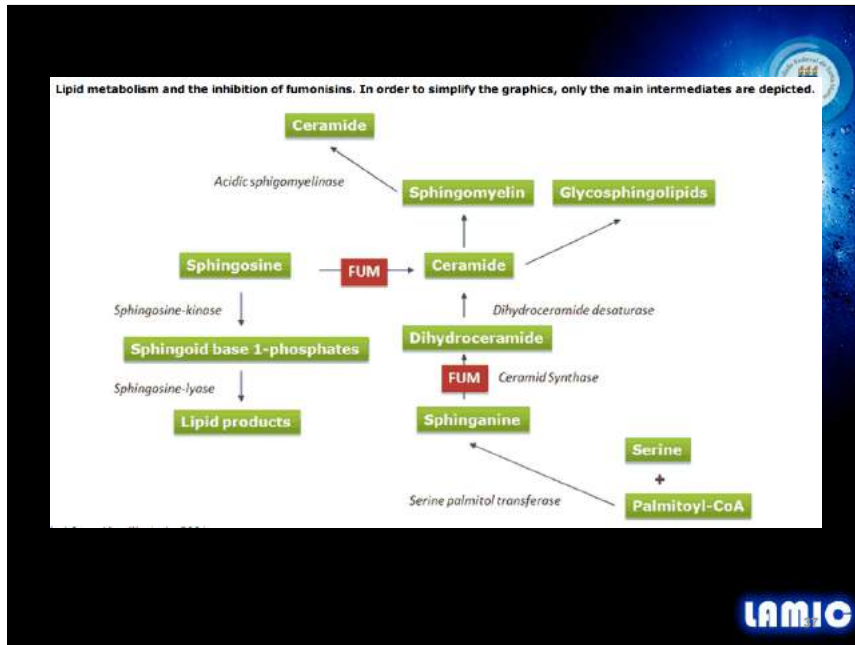
T-2 Toxina

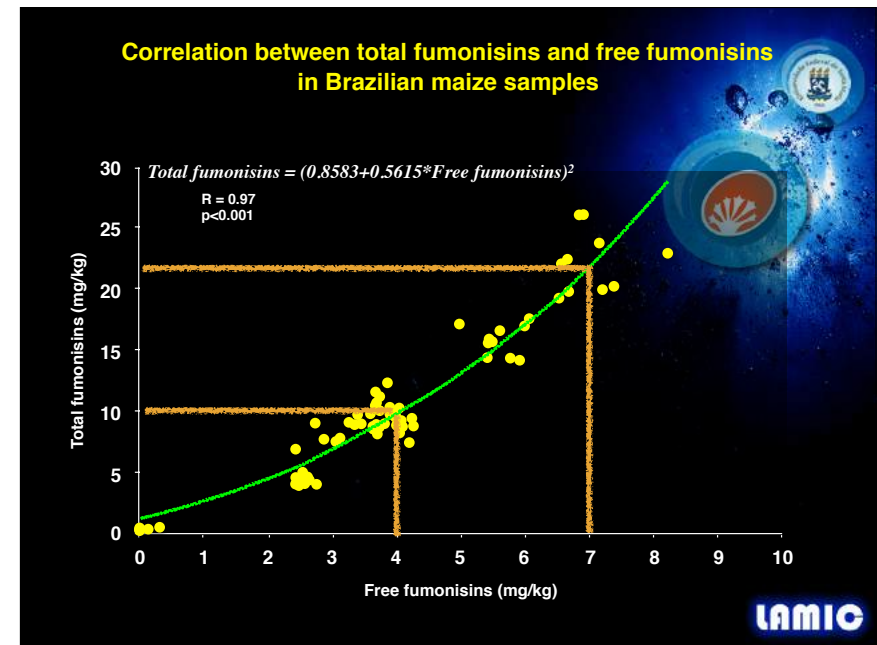
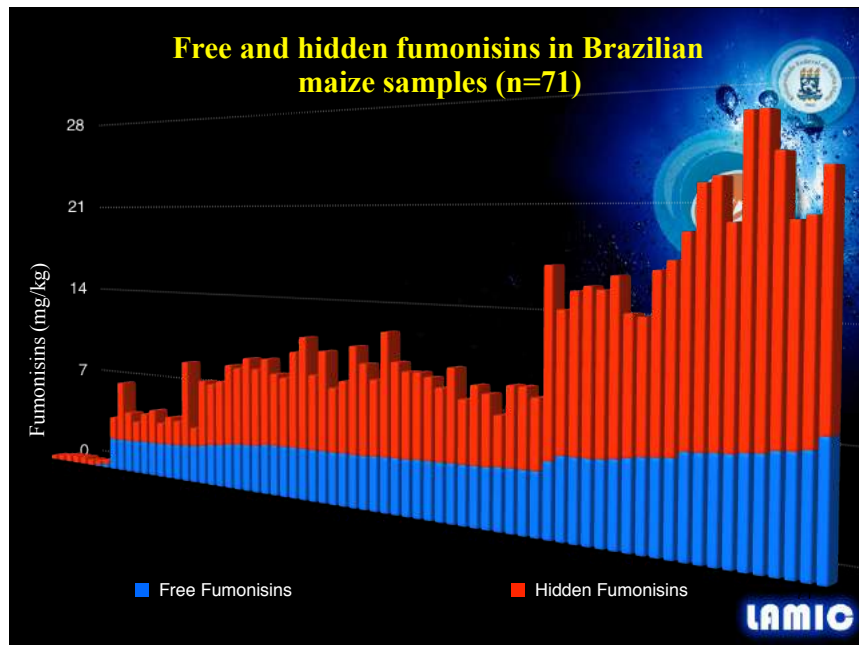
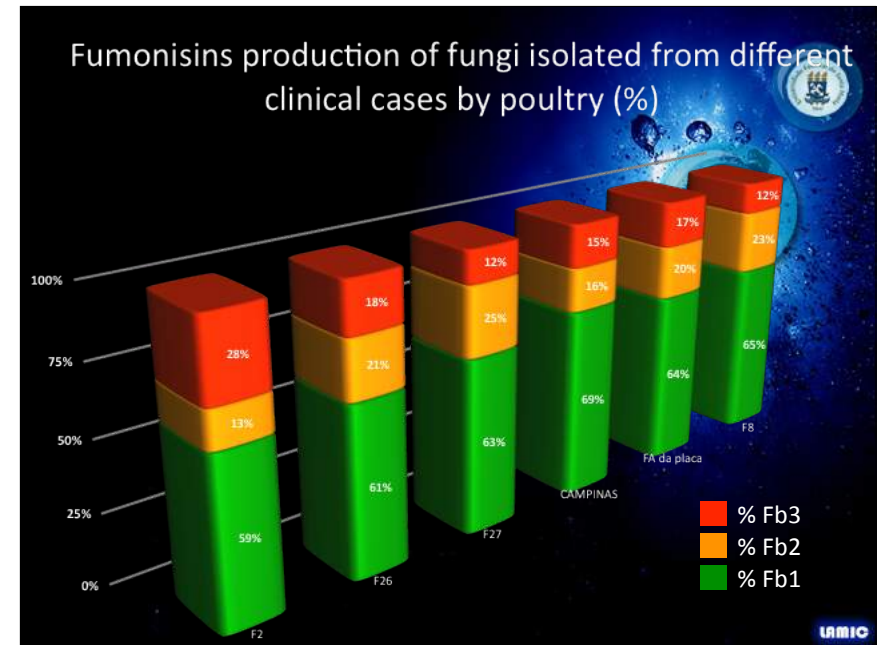
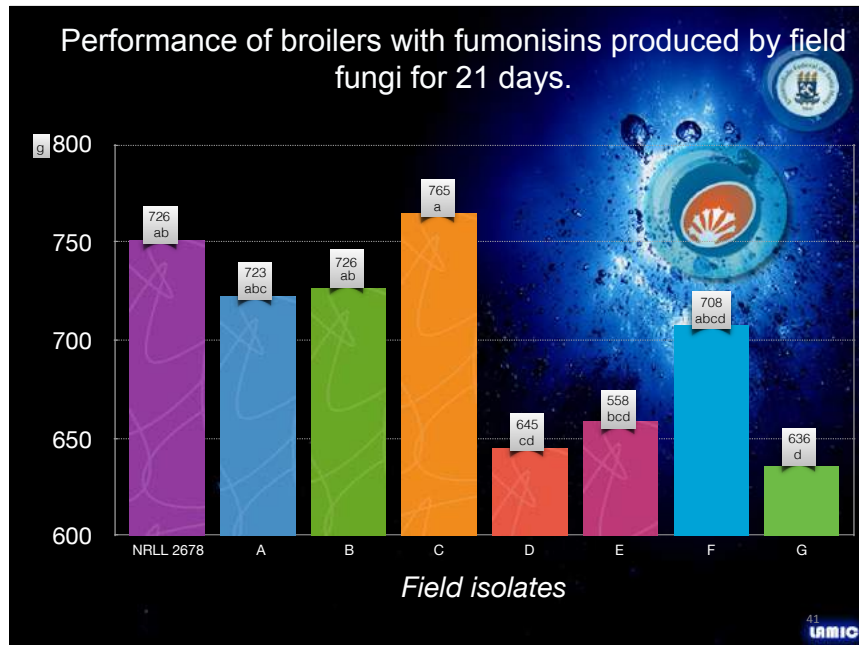


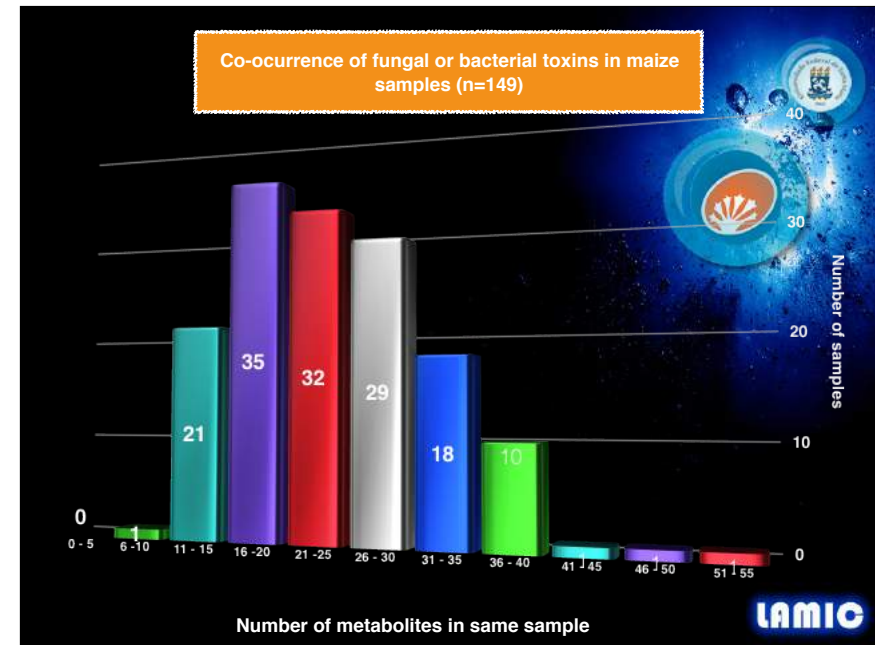
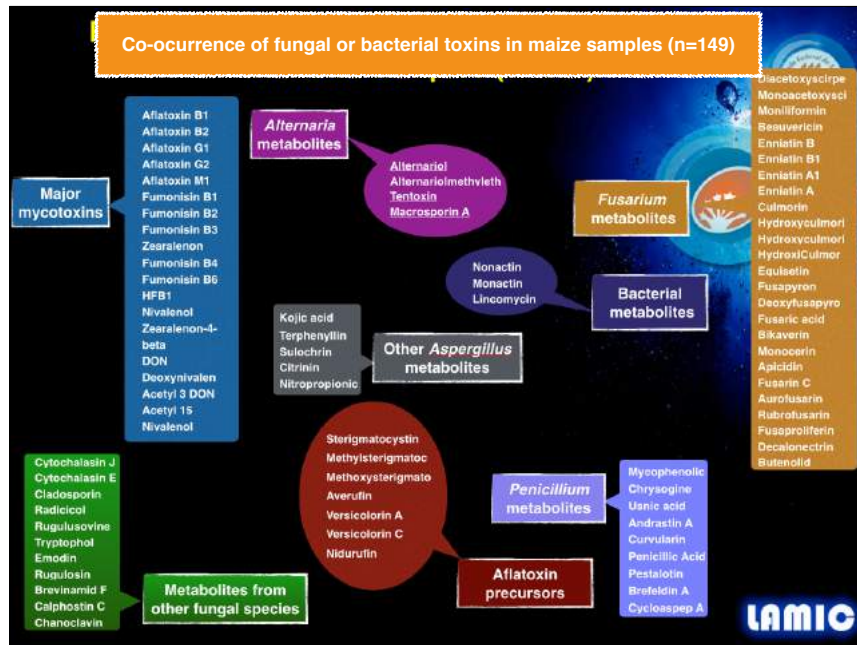
Zearalenone

FUMONISINS









TRICHOHECENES

T-2 Toxin and HT-2 Toxin

Deoxynivalenol (DON), 3Ac-DON, 15Ac-DON

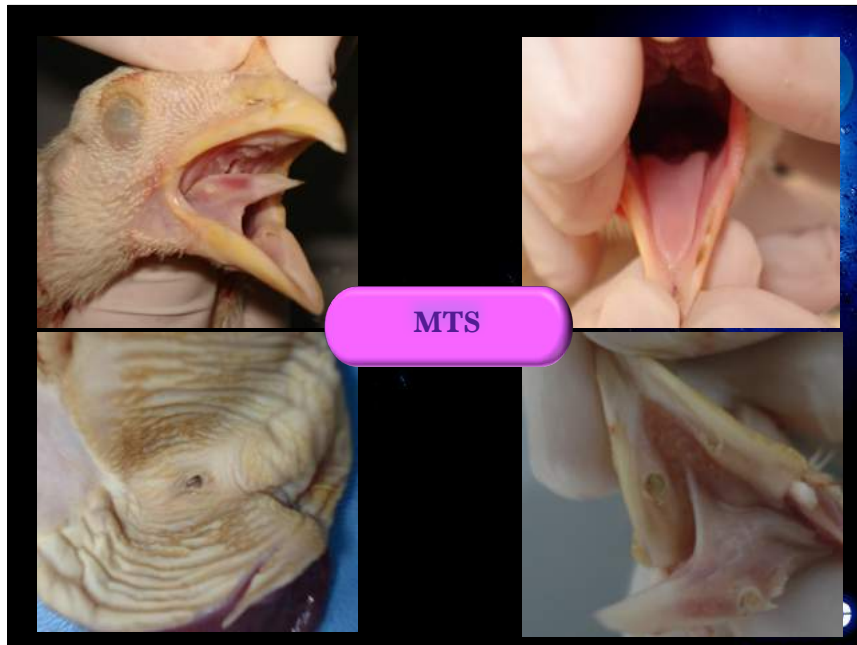
Diacetoxyscirpenol (DAS)

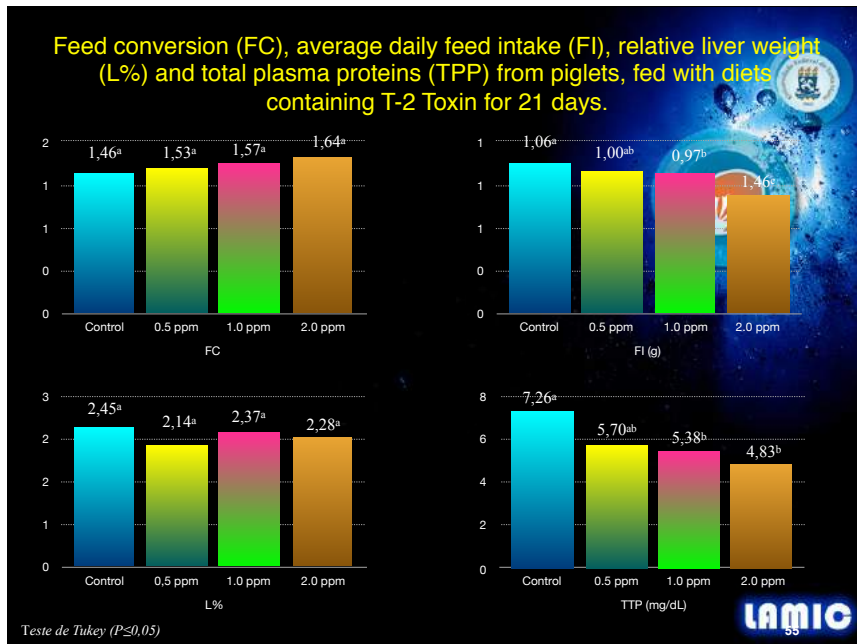
Fusarenon X

Nivalenol.....

F. sporotrichioidis

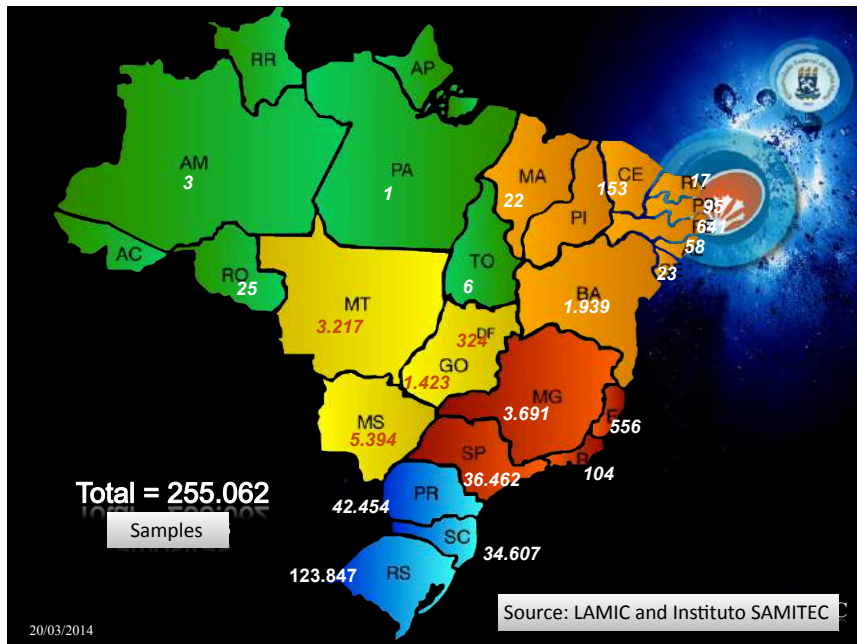
LAMIC





Prevalence of mycotoxins

LAMIC



Most important mycotoxins in Brazil

Toxin	Samples	Prevalence (%)	Average (µg/kg)	Average of positive (µg/kg)
Aflatoxins	175,027	38	8.2	71
Zearalenone	148,358	37	108	288
Fumonisin	84,820	72	1,656	2,290
Deoxynivalenol	69,422	47	400	852
Ochratoxin A	49,047	4.5	0.7	53
T-2 Toxin	18,827	1	2.4	1,141
DAS	3,531	3.3	3.7	3.7
3-DON	507	10.8	4.2	4.3
15-DON	728	12.2	3.8	3.8

n = 1,16 million of analyzes

Source: LAMIC and Instituto SAMITEC

Mycotoxigenic interactions - last 10 years (LC-MS/MS)

LAMIC & Instituto SAMITEC

Mycotoxins	n	Prevalence %	Mean 1	Mean 2	Mean 3
FBs + Zea	40,347	40	2,696	198	--
Afla + FBs	54,268	24	15	3,116	--
Don + Zon	46,108	20	1,130	404	--
Afla + Zon	36,679	19	4	118	--
Afla + Zea + FBs	36,862	14	10	174	3,357
FBs + Don	24,729	13	2,002	474	--
Afla + Don	33,677	4	9	468	--
Afla + Zea + Don	30,083	3	8	278	506

165,389 samples

58% of samples with one or more mycotoxins

MYCOTOXINS ON ANIMAL HEALTH AND WAYS TO EVALUATE ANTIMYCOTOXIN ADDITIVES

✓ BACKGROUND AND APPROACH

✓ MYCOTOXINS ON ANIMAL HEALTH

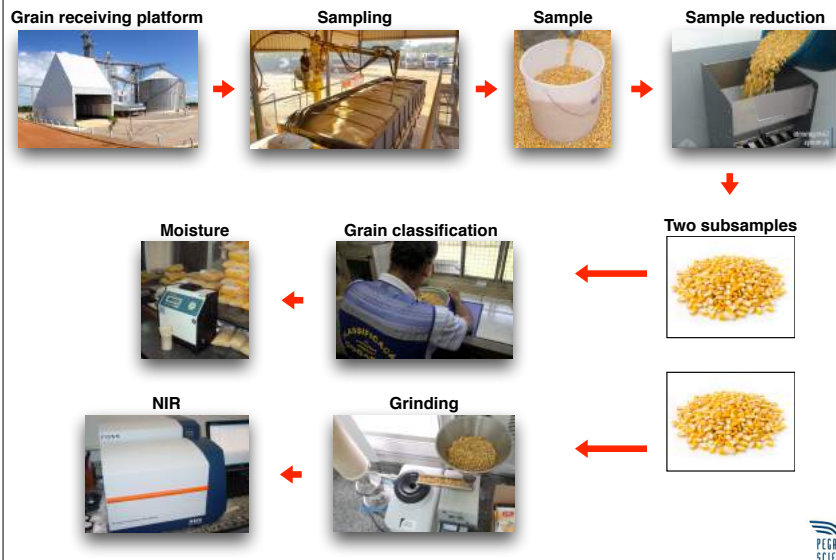
- Most important mycotoxins
 - Effects on animals
 - Clinical
 - Economical

✓ State of Art

✓ WAYS TO EVALUATE AntiMycotoxins Additives (AMA's)

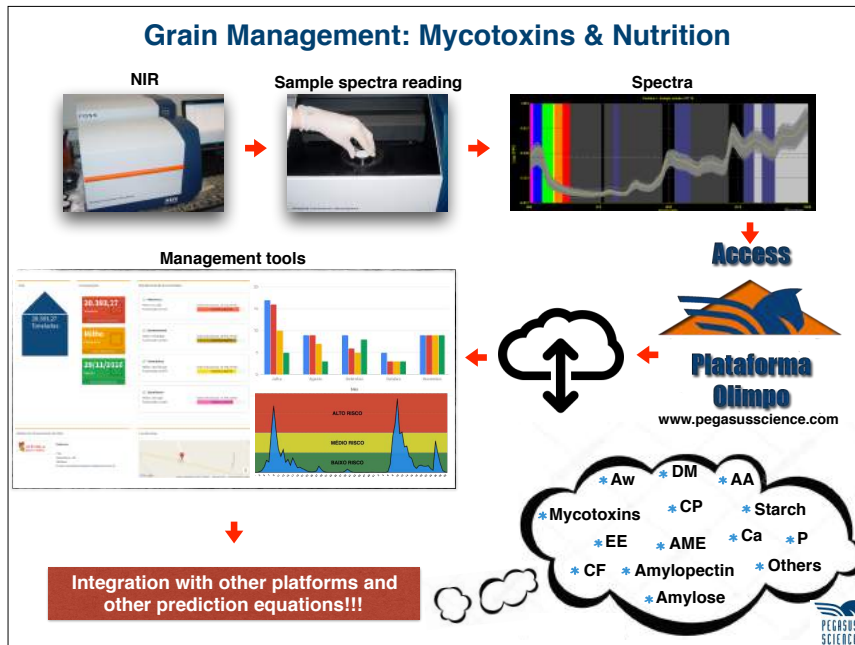
- *in vitro*
- *in vivo*
- Results

Grain Management: Mycotoxins & Nutrition



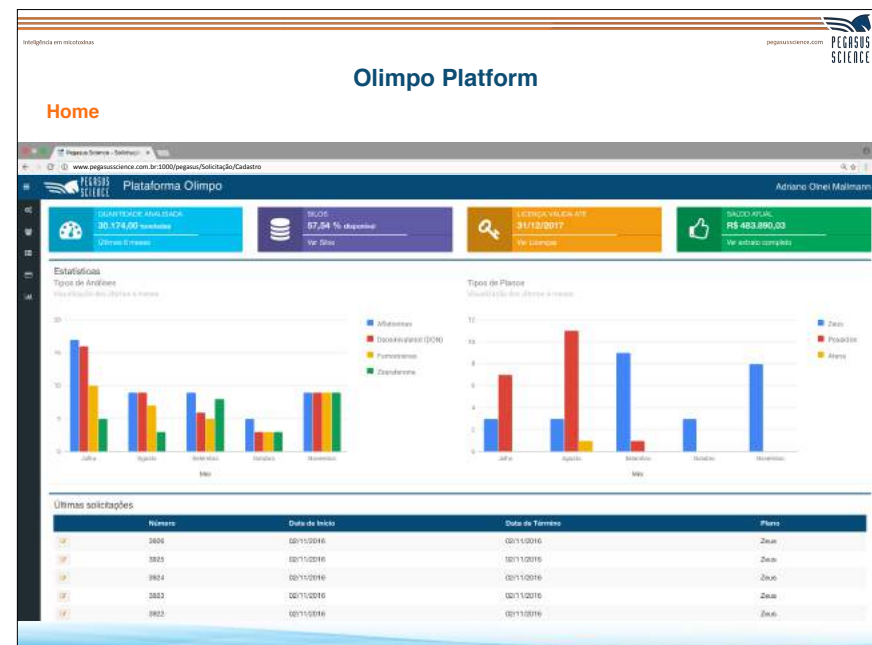
System calibration: Sample origin





Analyzes available for NIR

Raw materials	Analyzes available for NIR			
	DON Deoxynivalenol	ZEA Zearalenone	AFLA Aflatoxins (B ₁ +B ₂ +G ₁ +G ₂)	FUM Fumonisins (B ₁ +B ₂)
Maize				
Barley				
Wheat				
Wheat bran				
Wheat flour				



Inteligência em micotóxicos

pegasus.com.br PEGASUS SCIENCE

Olimpo Platform

Sample registration and analysis request

www.pegasus.com.br/1000/pegasus/Solicitacao/Cadastro

PLATAFORMA OLIMPO João

Solicitação - Cadastro

Peçoso: Avícola Boa Vista (17.155.202/0001-99) Tipo da Solicitação: Armazenamento Plano: Zeús

Amostra: Amostragem Fabrica de Paqueta Quantidade (Kg): 40.000 Sacos (60 Kg): 666,67

Cidade: Santa Maria (Rio Grande do Sul - Brasil) Milho: X163791

Análises da Amostra

Micotoxinas: Aflatoxinas, Deoxivalenol (DON), Fumonisinás, Zearalenona

Classificação Macroscópica da Amostra

Critério de classificação	Valor (%)
Umidade	12,00
Quebrados	
Impurezas	0,20
Clumachadas	
Avariados	
Ardis	

Descrição: Amostra 217

Botões: Voltar, Novo, Gravar, Enviar para Análise, Excluir

Inteligência em micotóxicos

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Olimpo Platform

Analysis report

Resultado

Avícola Boa Vista

Nº AMOSTRA: 3955 (161329) DATA DE INÍCIO: 30/10/2016 19:32:16 DATA DE TÉRMINO: 30/10/2016 19:51:33 MATRIZ: Milho PLANO: Zeús

DESCRIÇÃO: Amostra 1109

Micotoxina	Resultado (ppb)	Classificação Macroscópica	Valor (%)	Bonificação/Desconto	Valor (%)
Aflatoxinas	11	Umidade	12,00	Aflatoxinas	-1,00
Deoxivalenol (DON)	ND	Quebrados	1,00	Impurezas	2,00
Fumonisinás	2067	Impurezas	0,20	Fumonisinás	2,10
Zearalenona	ND	Clumachadas		Impurezas	-0,20

55.000,00 kg PESO INICIAL 1.595,00 kg +2,90 % BONIFICAÇÃO 56.595,00 kg PESO FINAL

Resultados de análise de micotoxinas emitidos pela Pegasus Science utilizando metodologia NMR. A classificação da amostra e os critérios de bonificações e descontos são de responsabilidade do(a) Avícola Boa Vista.

Impresso em 04/11/2016 16:41:21

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Inteligência em micotóxicos

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Olimpo Platform

Management of silos

Armazenar no Silo

Silo	Capacidade total	em uso	Disponibilidade
Silo 02 - Milho	5.000 (t)	0,00%	5.000 (t)
Silo 04 - Milho	1.000 (t)	2,50%	975 (t)
Silo 01 - Trigo	1.304 (t)	5,27%	1.236 (t)
Silo 07 - Milho	3.000 (t)	85,51%	456 (t)

Botões: Armazenar, Ver situação

Inteligência em micotóxicos

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Olimpo Platform

Management of silos

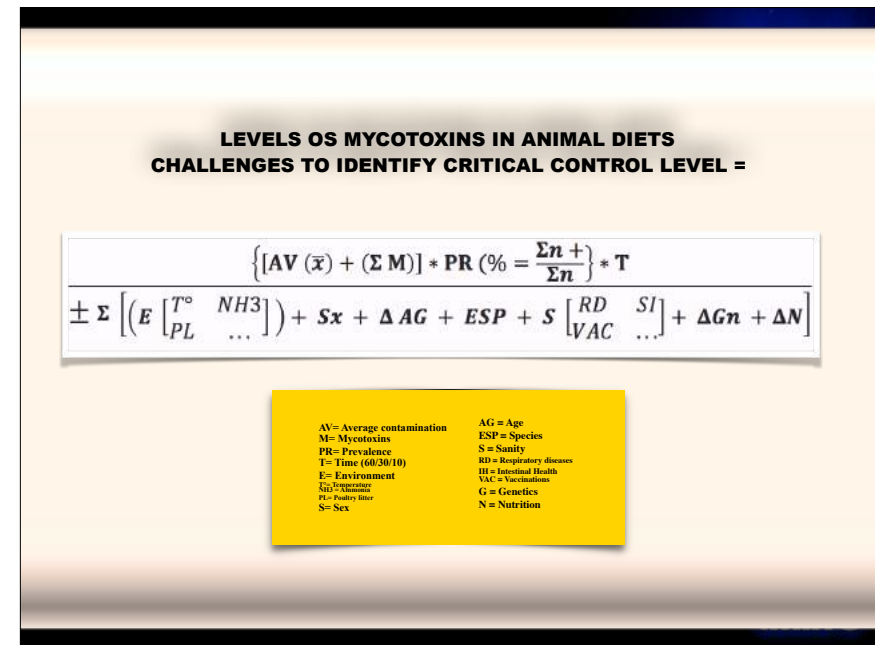
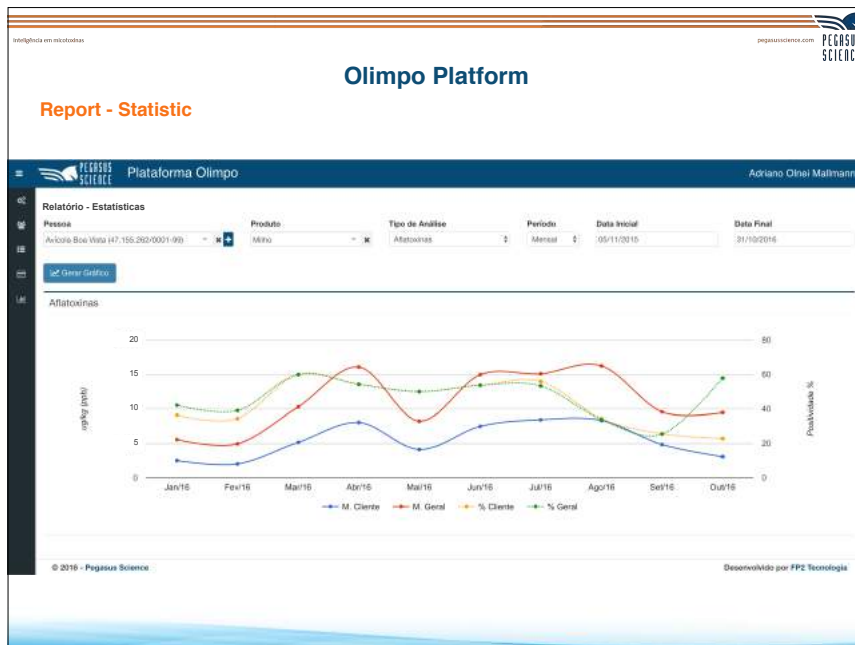
Armazenar no Silo

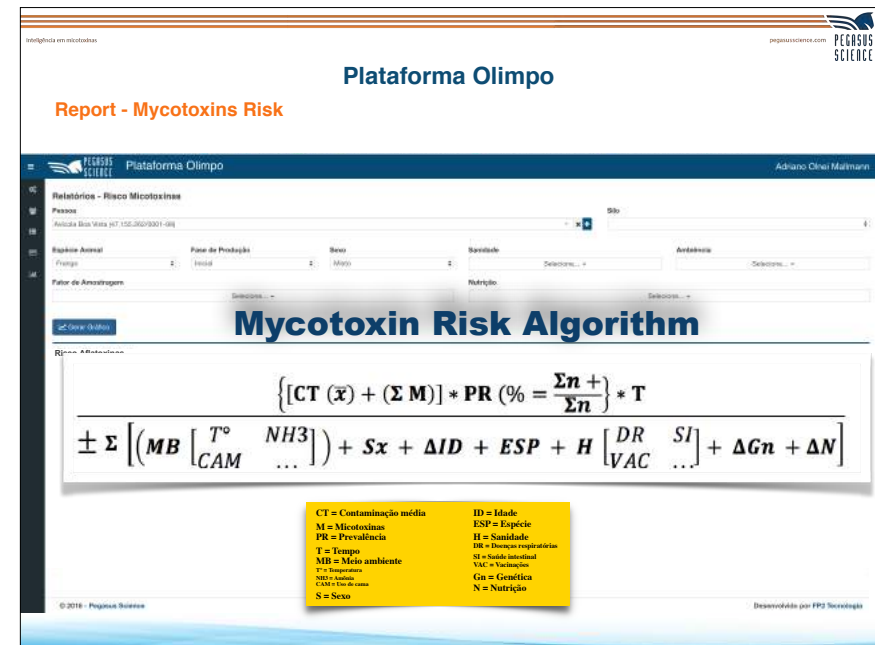
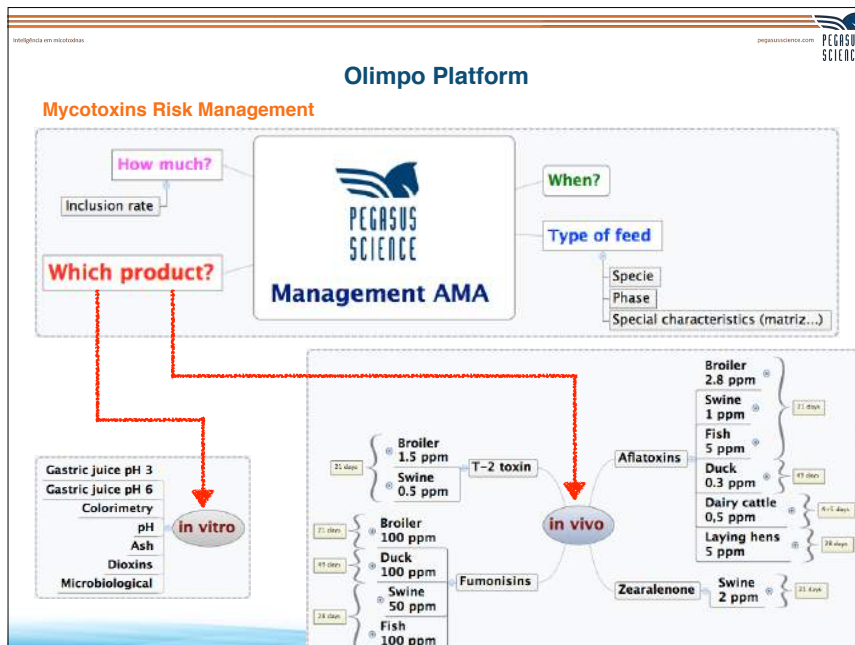
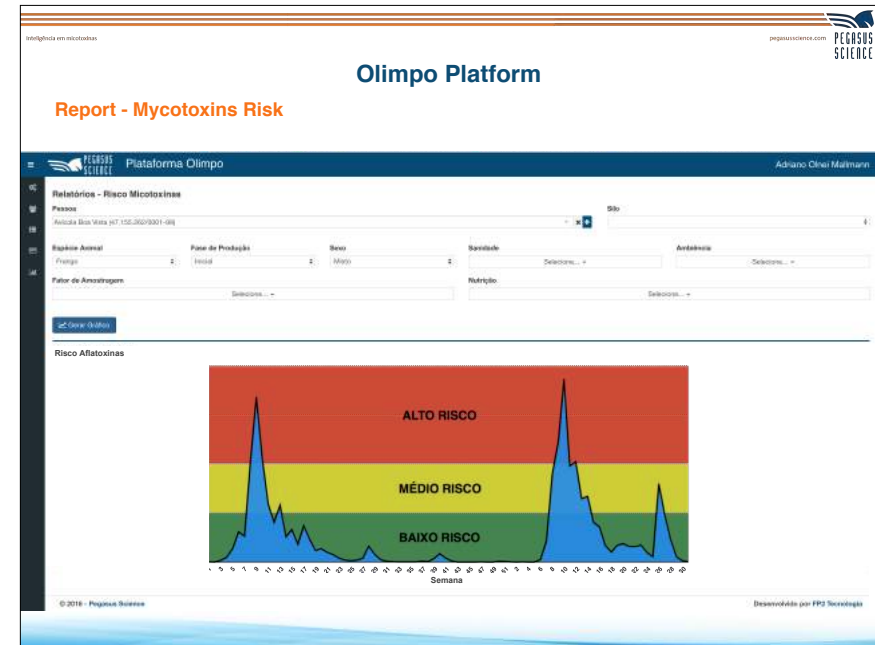
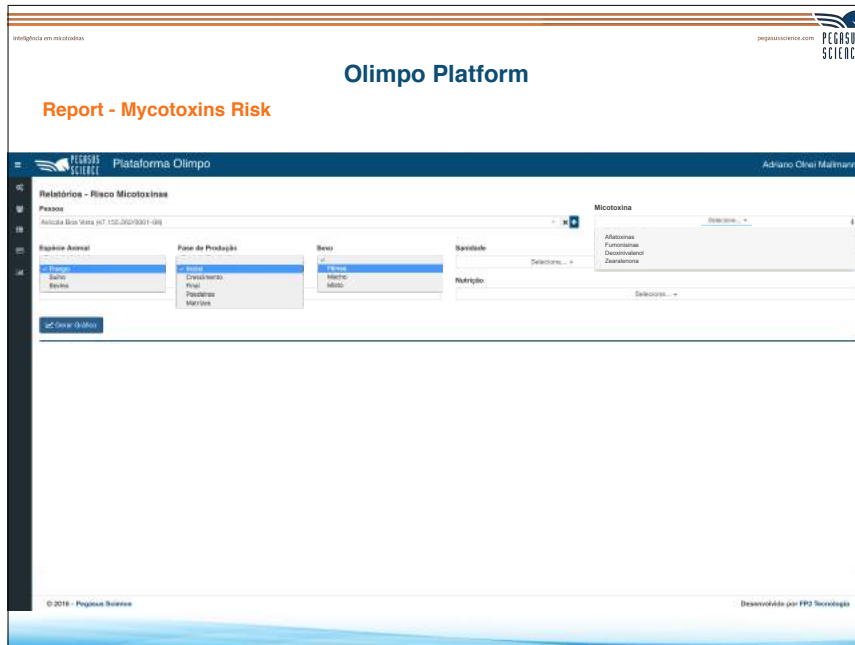
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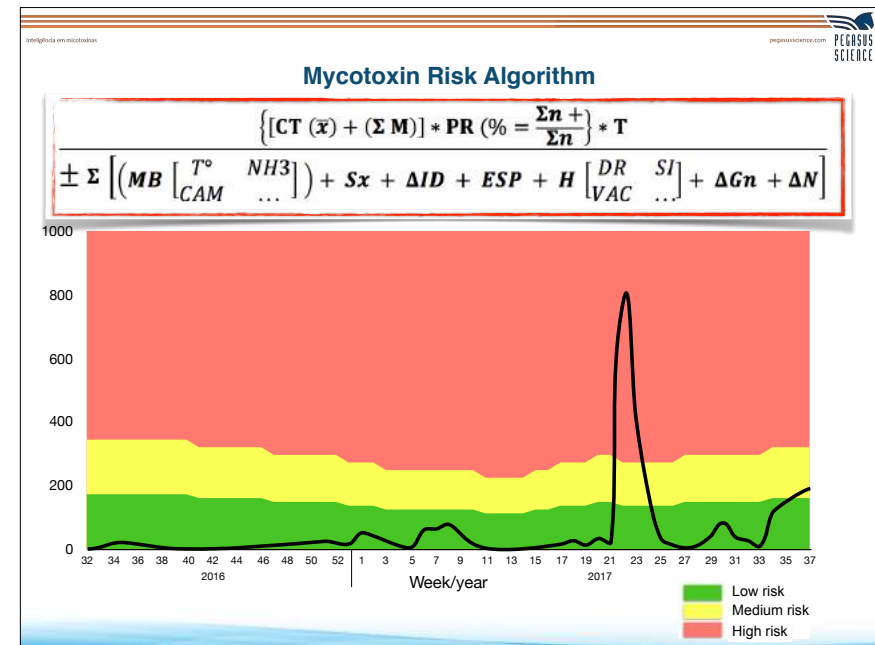
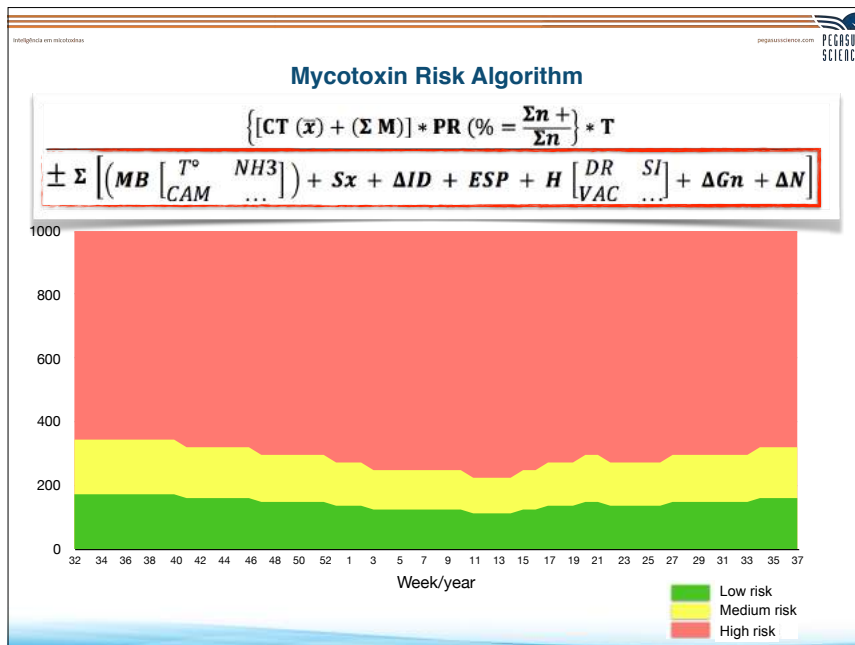
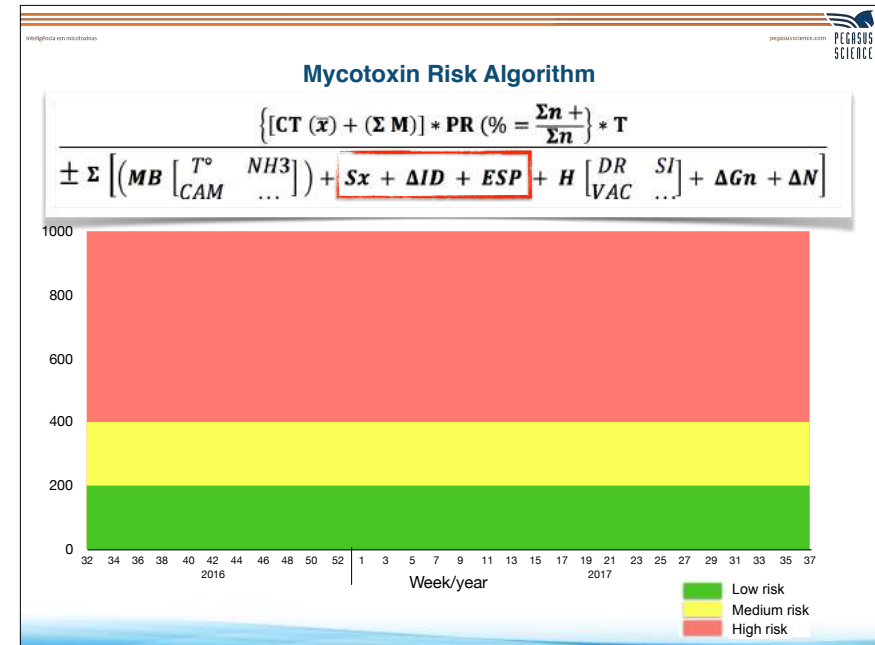
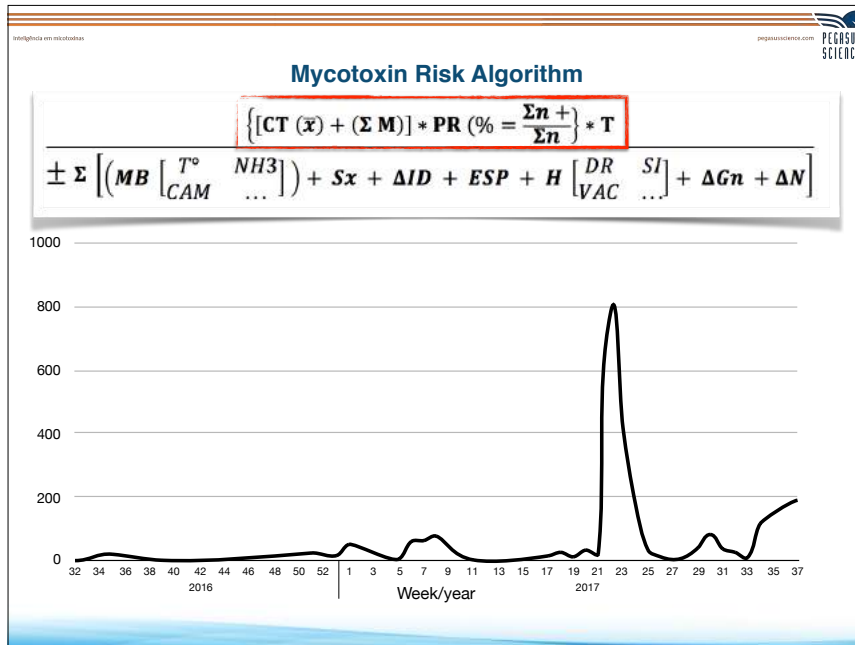
Botões: Armazenar, Ver situação

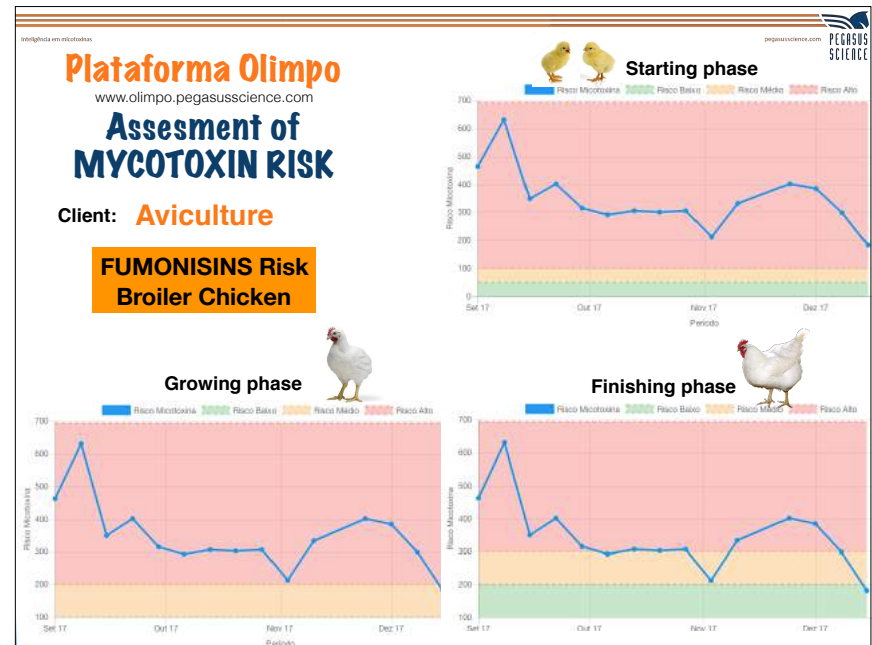
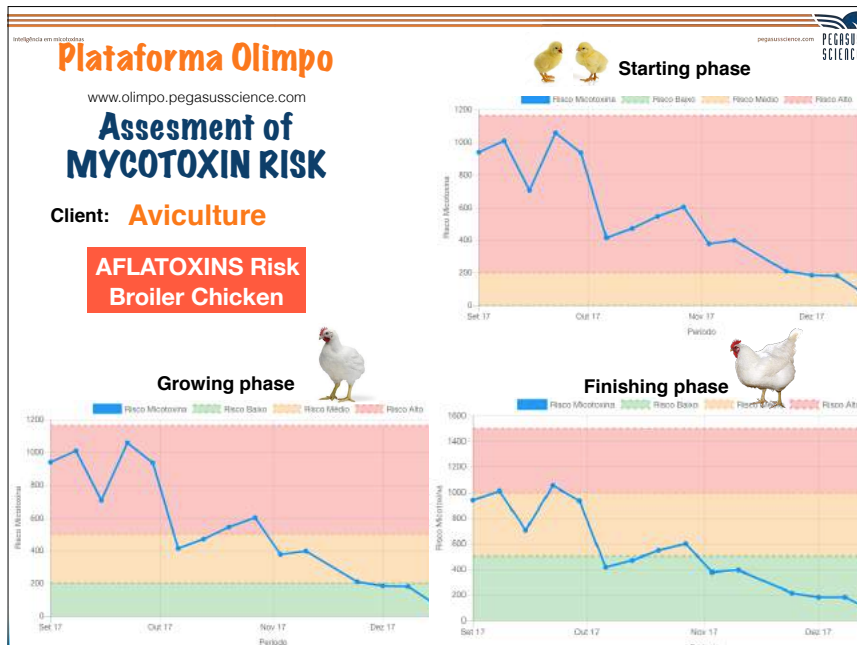
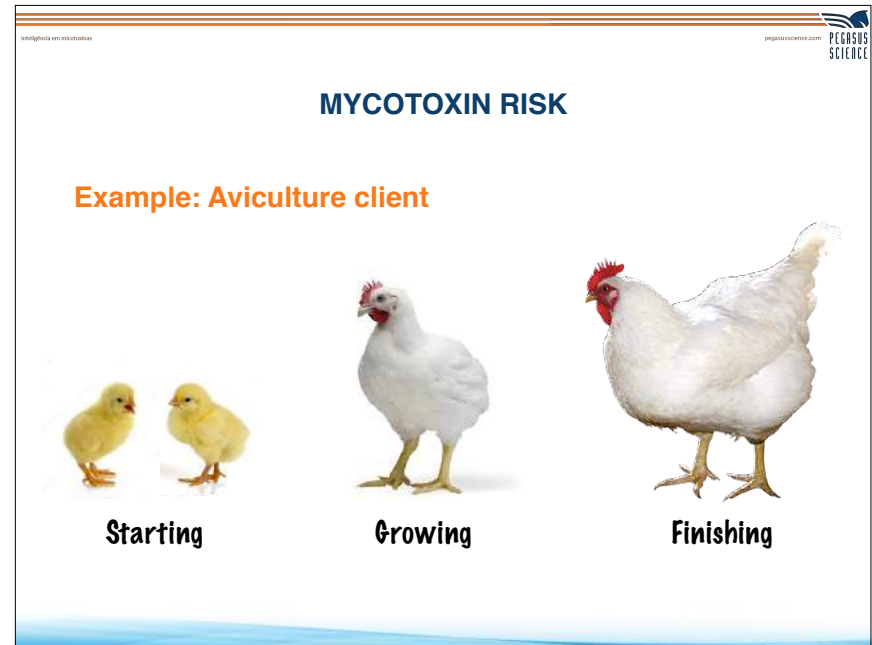
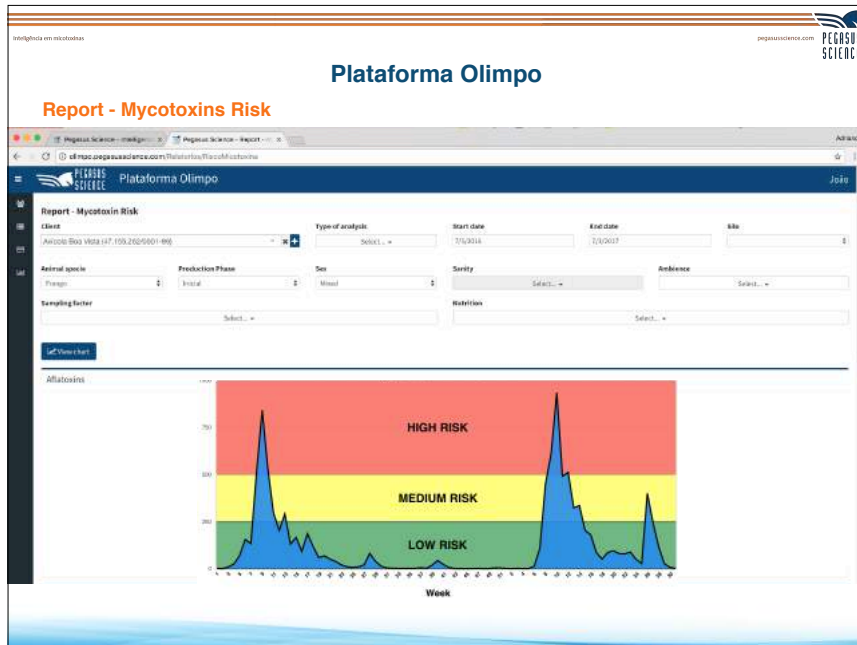
Informações de amostras:

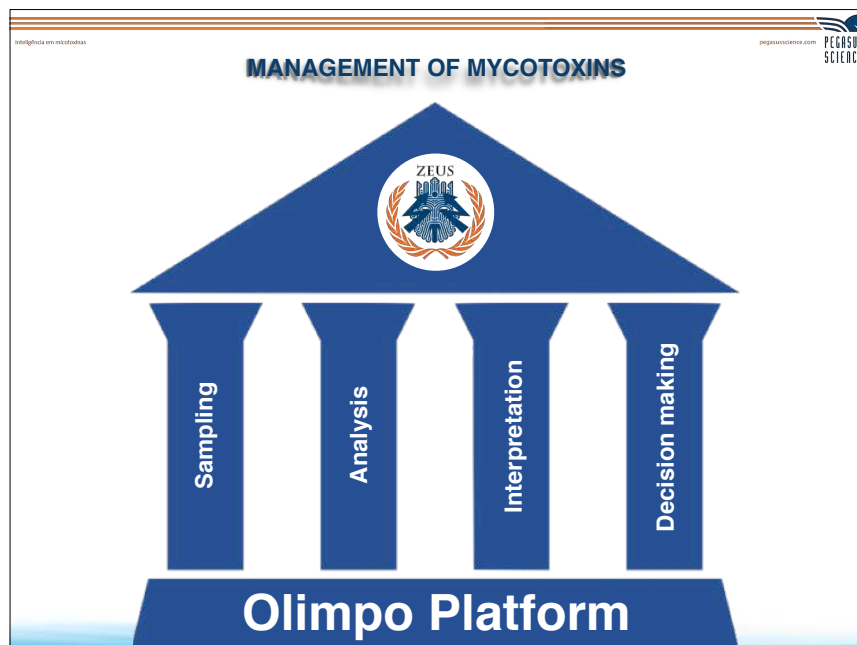
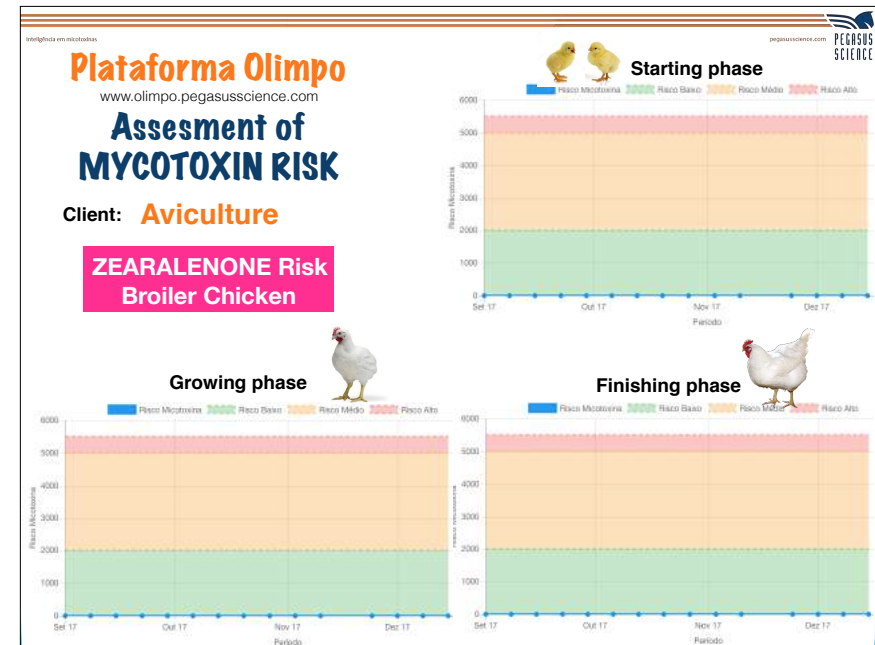
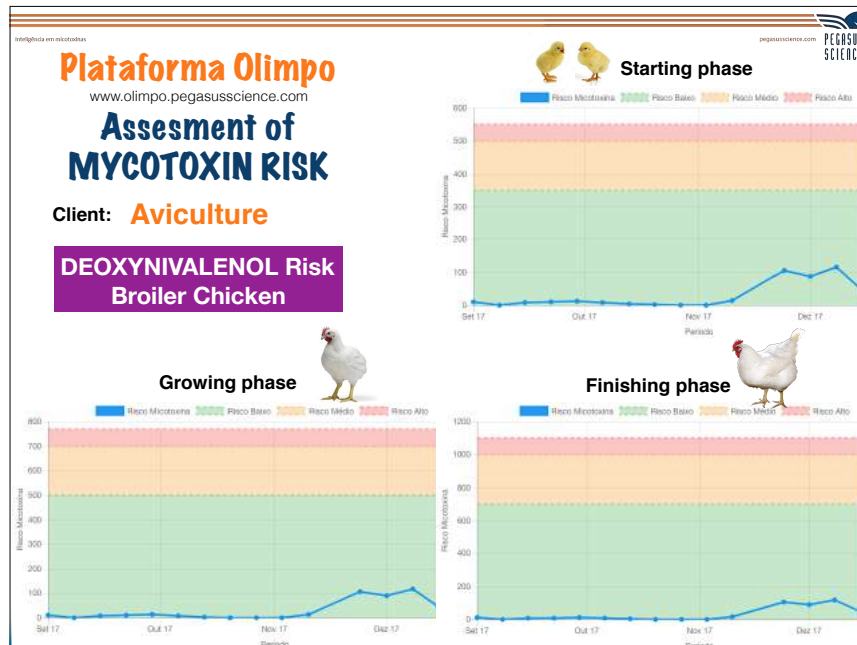
- Aflatoxinas: Valor analisado: 2.317,868 ppb
- Deoxivalenol (DON): Valor analisado: 2.303,217 ppb
- Fumonisinás: Valor analisado: 2.063,207 ppb





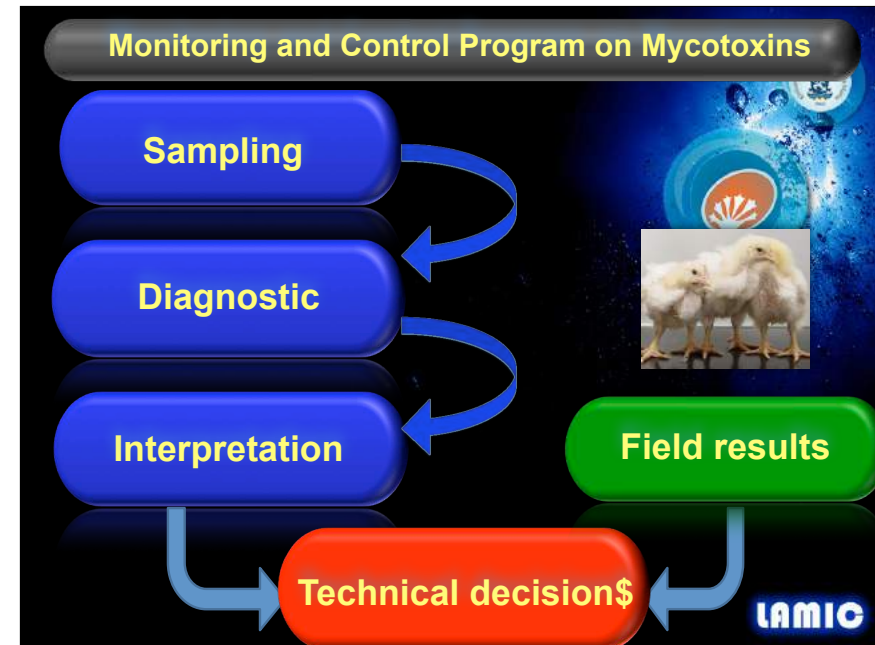






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Management and Control of Mycotoxins



Monitoring and Control Program on Mycotoxins

Sampling



- ## Mycotoxins: a ppb's and ppm's problem
- How much does 1 billion (1,000,000,000) grains of maize weight?
 - One day has 86,400 seconds. How long would we gotta live in seconds to live 1 billion of seconds?
 - A 30 day month has 2,592,000 seconds. 10 ppm of this month passed while you read it.
- 

What is the weight of 1 billion (1,000,000,000) grains of corn?



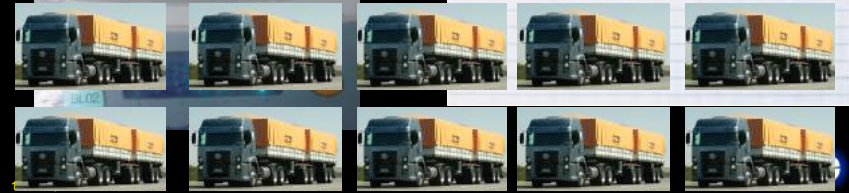
LAMIC

How much does 1 billion (1,000,000,000) grains of maize weight?

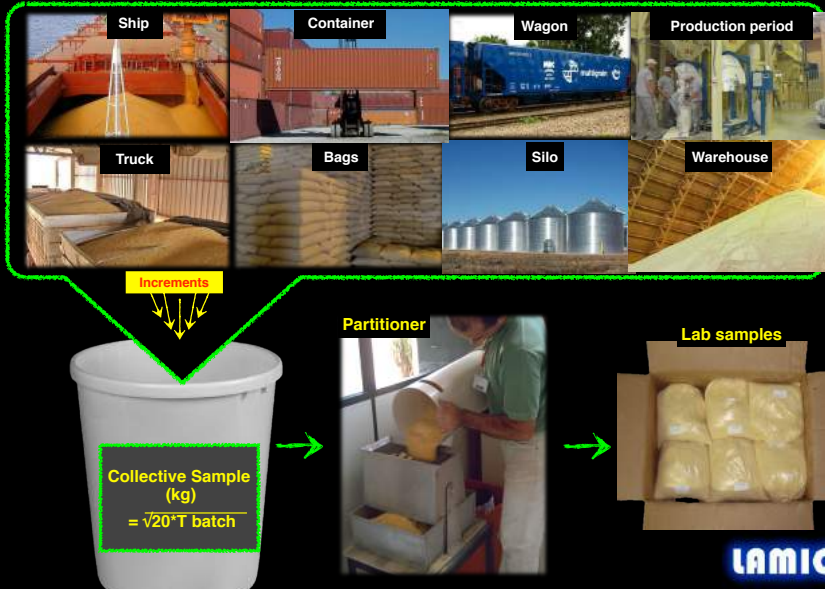


1 grain = 0,3512g
 x 1.000.000.000

 351.200.000 g
 351.200 Kg
351,2 T



Sampling process for mycotoxins



“HOLE IN THE SCREW CONVEYOUR”

Bulk products (LAMIC)

$$\sqrt{20} \times T$$



Kg of collective sample



Ex: 500 T / day = 100 Kg



Laboratory (1 Kg)

LAMIC



LAMIC + INSTITUTO SAMITEC

- 2 U-HPLC MS/MS (LC MS/MS).
- 5 HPLC MS/MS (LC MS/MS).
- 1 HPLC MS.
- 5 HPLC FLD.
- 1 HPLC UV.

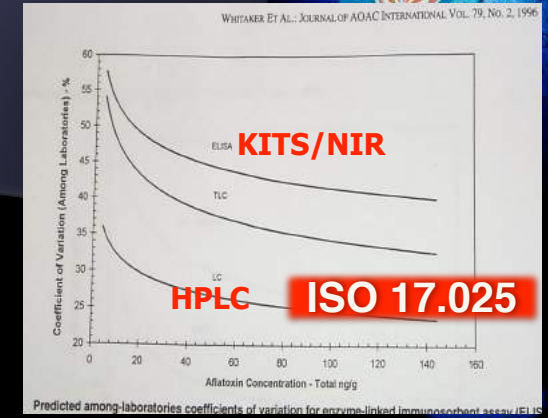
Total 14 LC USED FOR MYCOTOXINS DIAGNOSTICS



Analytical Methods

- Thin-layer chromatography (TLC)
- Immunoassays (ELISA Kits)
- HPLC (-UV, -FLD)
- GC (-FID, -ECD, -MS)
- LC-MS, LC-MS/MS

NEW NIR!!!



Monitoring and Control Program on Mycotoxins

Solutions

LAMIC

Solution #1

LAMIC

Classification in the gravity table separator

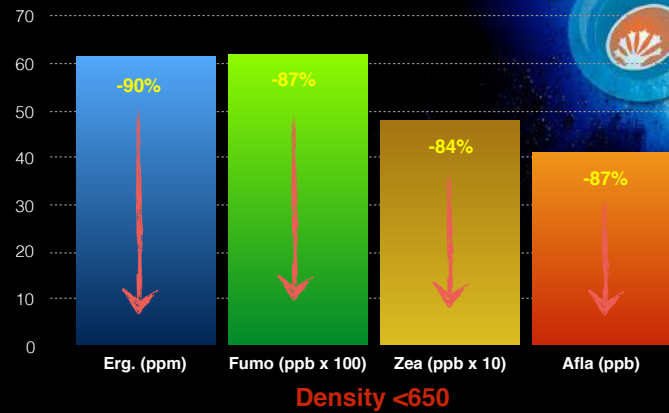


LAMIC



LAMIC

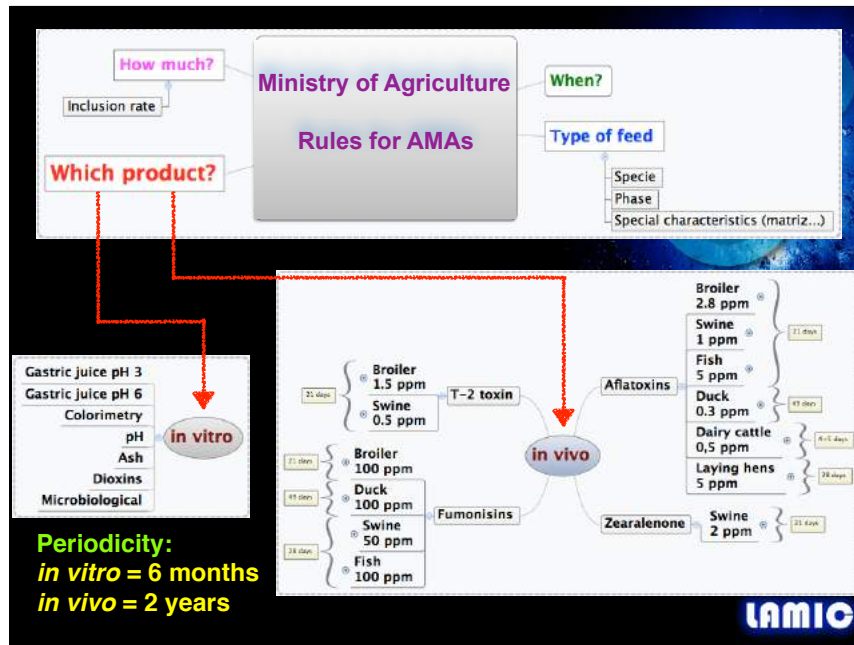
Impact of pH (>7.50 and <6.50) mycotoxin contamination on corn



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Solution #2

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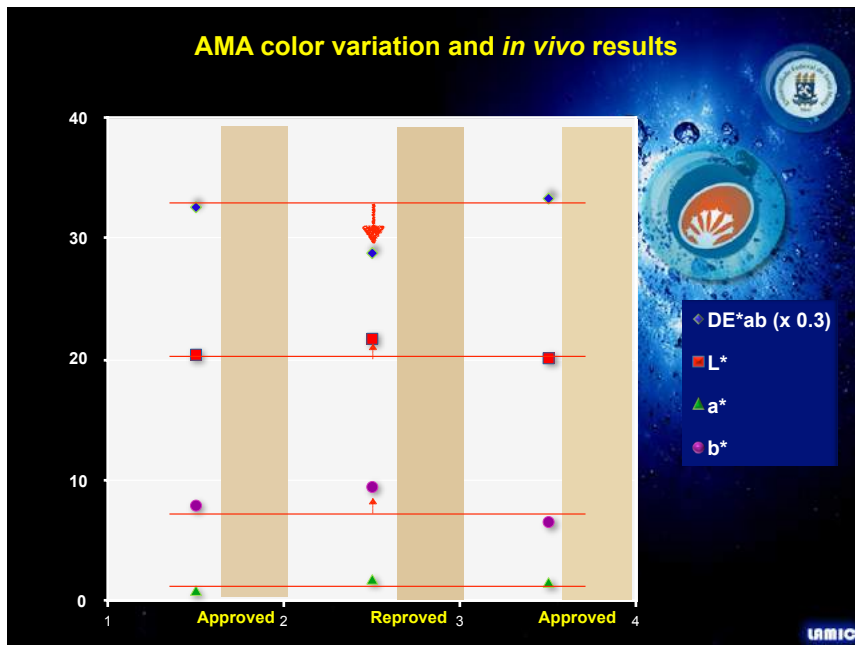
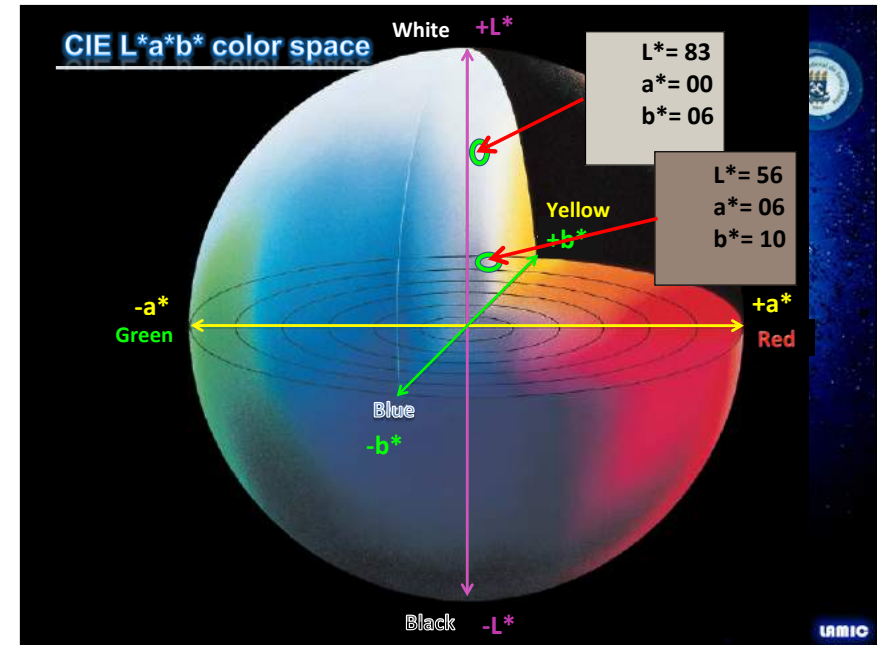


> 1,000 samples

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in vitro

IIC



Colorimeter to measure the color and control of AMA

Laboratório de Análises Micotoxicológicas
Universidade Federal de Santa Maria - CCR & CCS - DMVP & DIAC

Report of analysis of adsorption *in vitro*

Requester: Nonono Date: 01/01/2010

Sender: Nonono Responsible: Nonono

Substrate: toxin = 5000:1 (5000 ppm/1000 ppb).

PRODUCT	% CONCENTRATION	% ADSORPTION / INACTIVATION
Aflatoxina B1	0.50	95,80 (±1,98)

Magrodo de adsorção/inativação em pH 3,0 - inativação B1

CONCLUSION

The antimycotoxin additive **Nonono** (color L*=56.06, a*=6,28, b*=11,61, ΔE*ab=45,5) has a coefficient of adsorption/inactivation *in vitro* of 95.80% (ninety five point eighty percent) for Aflatoxin B1 (1.0 µg/mL) in artificial gastric juice, pH3.0, and inclusion rate of 0.50%. **+ pH and ash.**

ADSORPTION CONDITIONS / INACTIVATION
Triplicate samples of artificial gastric juice at pH 3.0, as described in Pharmacopeia National Formulary - USP XXII (1990), at a concentration of 1.0 µg/mL.

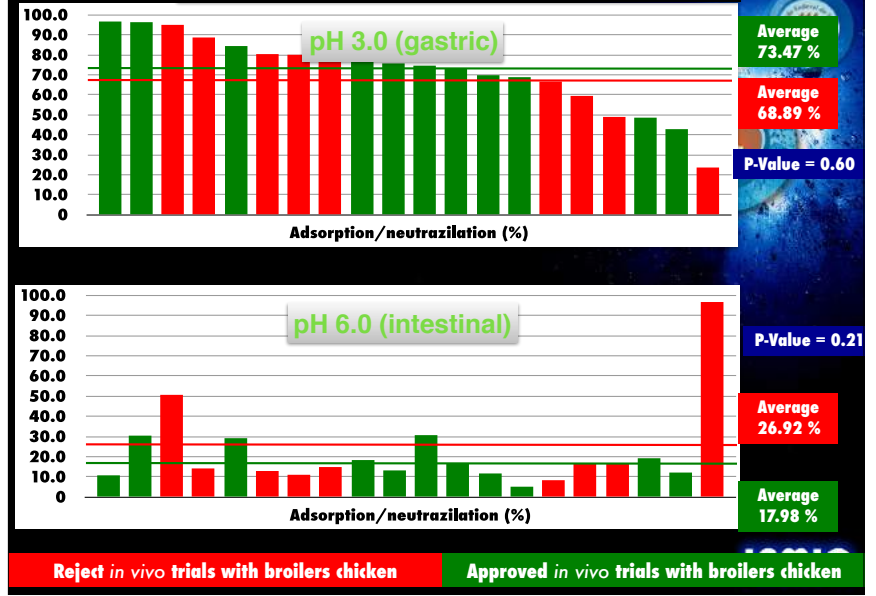
R=0,9787 P=9,92
Linear Equation: Adsorption / inactivation = 72,21 + 52,92*(Dose Linear Simple Regression)

EQUIPMENT REVIEW
Automated system with pre-column derivatization with ASPEC XL4. Liquid chromatography mass spectrometry (LC / MS / MS). Interpretation and analysis of data: System ChemStation Agilent™.

CONCLUSION:
The additive anti-mycotoxins **Nonono**, white color of L*=56.06, a*=6,28, b*=11.61, and ΔE*ab=45.5, has a coefficient of adsorption/inactivation *in vitro* of 95.80% (ninety five point eighty percentage points), compared to the standard of aflatoxin B1 (1.0 µg/mL) in artificial gastric juice, pH 3.0, and inclusion rate of 0.50%.

Why we need an "in vivo" evaluation?

Correlation pH 3.0 x pH 6.0 (20 products)



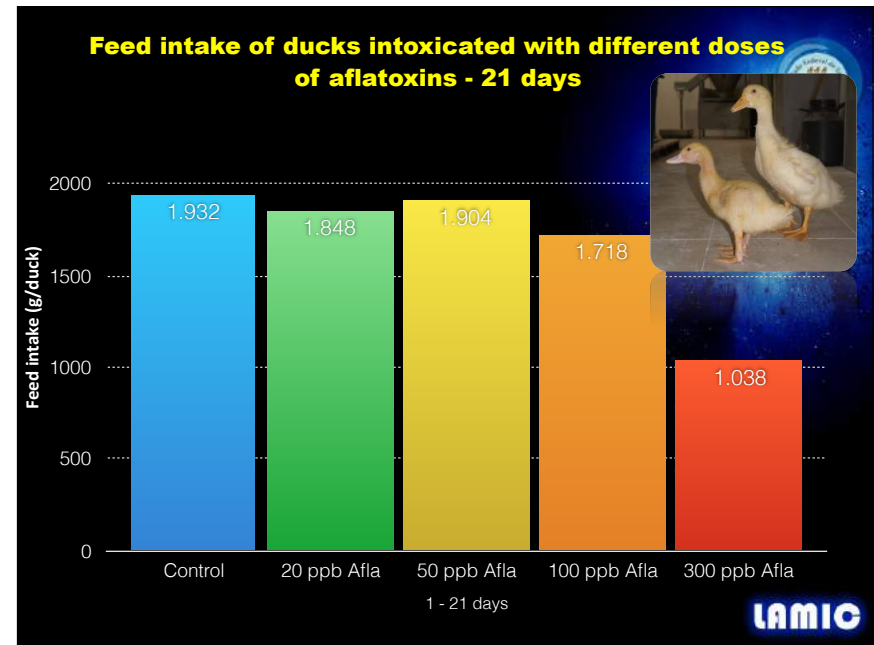
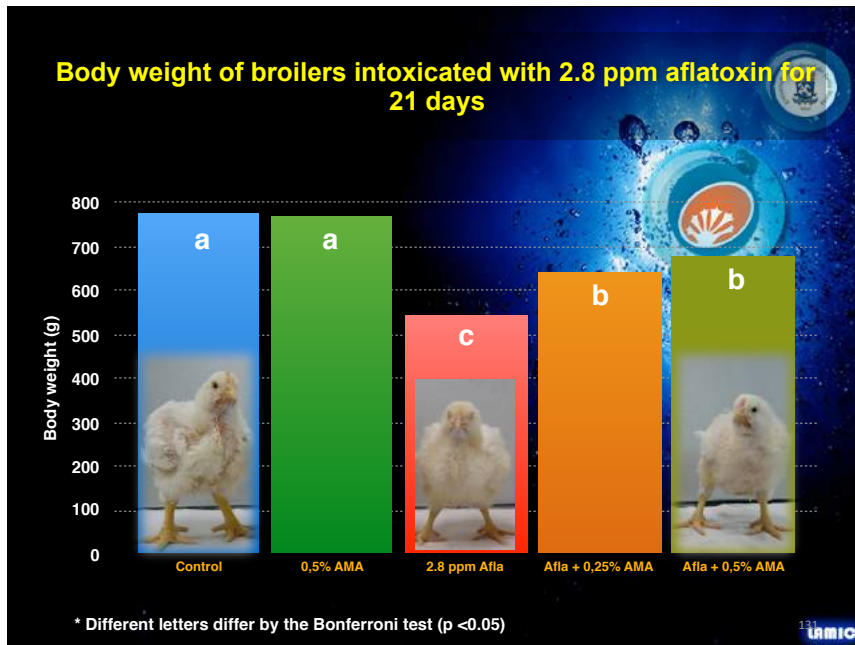
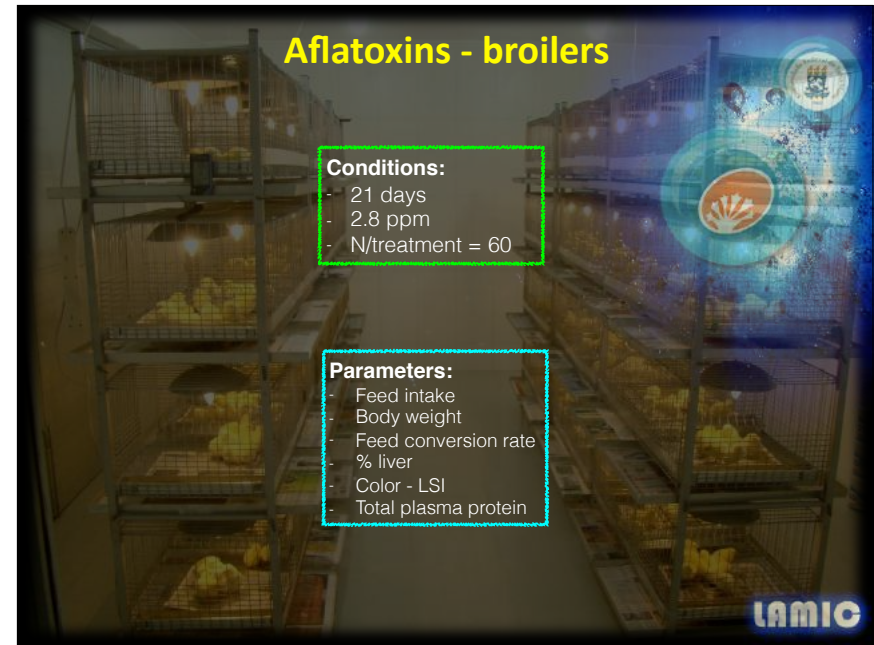
Protocol for *in vivo* experiments

TREATMENT	Toxin (ppm)	AMA (%)
1	0	0
2	0	X
3	X	0
4	X	X
5...	X	X/2...

in vivo

ISO 9001: 2008





Aflatoxins + Fumonisin + T2-Toxin: laying hens

Conditions:

- 21 days
- 3.0 ppm
- N/treatment = 60

Parameters:

- Feed intake
- Egg weight
- Feed conversion rate
- Color and shell thickness
- Color, thickness, blood and meat stains in egg white or yolk
- Haug units
- Total plasma protein

% of liver

Control

Body weight = 792 g
Liver weight = 20.3 g
Liver/body w. = 2.6%

Afla 2.8 ppm

Body weight = 585 g
Liver weight = 22.8 g
Liver/body w. = 3.9%

Color livers of chickens intoxicated with aflatoxins with and without AMA

CIE L*a*b* color space

White +L*

Black -L*

Green -a*

Red +a*

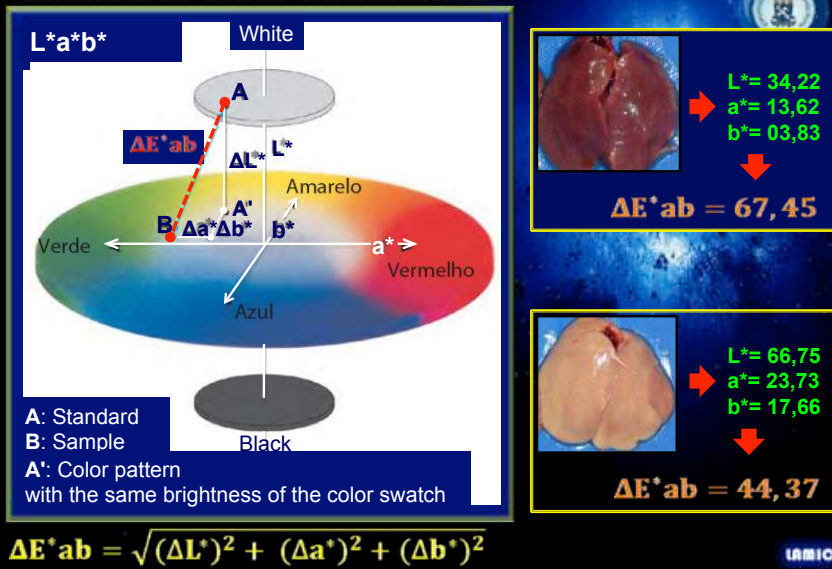
Blue -b*

Yellow +b*

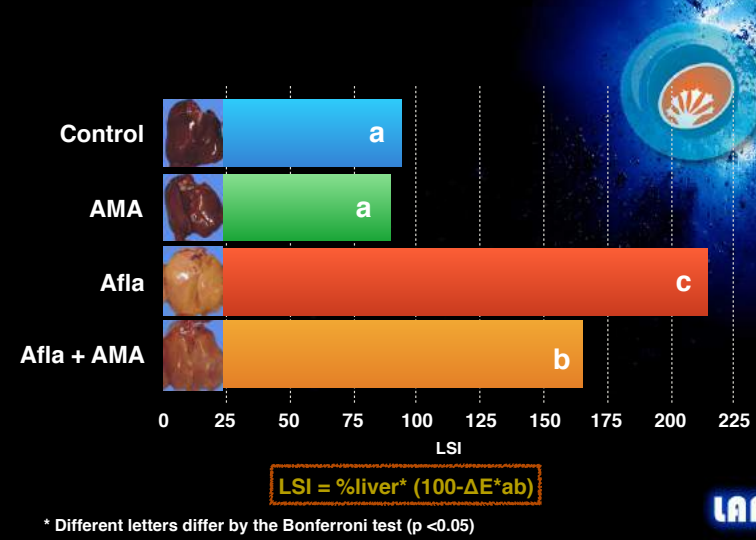
Inset 1: L* = 67, a* = 20, b* = 18

Inset 2: L* = 36, a* = 16, b* = 04

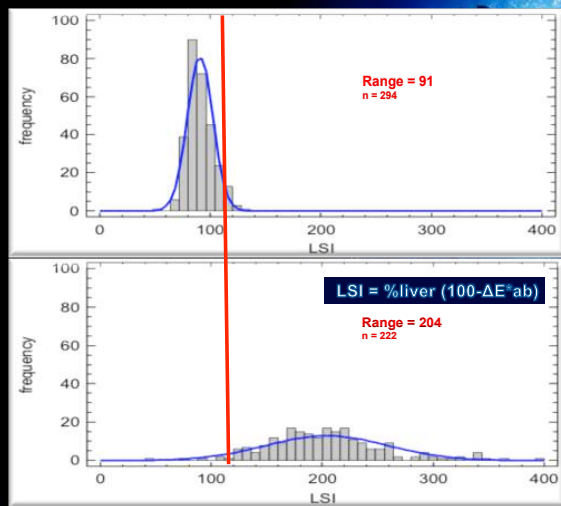
Measur of color differences



LSI = Color X % Liver of broiler chickens intoxicated with aflatoxins



Index of Colorimetric evaluation x % of liver broiler intoxicated with aflatoxins, 21 days.



Experimental unit: fish



Fumonisin: fish

Conditions:

- 21 days
- 5 ppm
- N/treatment = 60

Parameters:

- Body weight
- Length
- Liver histopatology



Control

5 ppm Fumo

Fumo + AMA

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TECHNICAL REPORT: EFFECTIVENESS OF THE ADDITIVE ANTI MYCOTOXIN [] IN DECREASE THE EFFECTS OF T-2 TOXIN ON CHICKENS

INSTITUTO SAMITEC
Instituto de Soluções Analíticas, Microbiológicas e Tecnológicas Ltda.
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www.samitec.com.br - falecom@samitec.com.br

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Table 5 – Body weight of the broilers fed with a diet containing T-2 Toxin, supplemented or not with [], for 21 days

Treatment	Initial		7 days		14 days		21 days	
	BW ¹	CV ²	BW	CV	BW	CV	BW	CV
Control	46.88 ^a	5.4	173.77 ^a	7.6	430.38 ^a	8.4	761.73 ^a	9.6
0.50% []	46.95 ^a	5.4	173.40 ^a	8.1	423.84 ^{ab}	8.2	753.07 ^a	8.8
2 ppm T-2 Toxin	46.77 ^a	5.5	165.08 ^b	7.8	411.46 ^b	7.6	706.94 ^b	9.3
2 ppm T-2 Toxin + 0.25% []	46.62 ^a	5.4	165.86 ^b	6.4	412.78 ^b	8.2	708.29 ^b	9.4
2 ppm T-2 Toxin + 0.50% []	46.60 ^a	5.9	170.90 ^{ab}	8.1	423.53 ^{ab}	8.6	749.03 ^a	9.7
Average	46.76	5.5	169.80	7.6	420.40	8.2	735.81	9.4

a – b = Averages in columns with different letters differ by the Bonferroni test (P<0.05).

¹BW = Body weight (g).

²CV = Coefficient of variation (%).

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SISTEMA DE GESTÃO CERTIFICADO
Fornecedor
NBR ISO 9001:2008

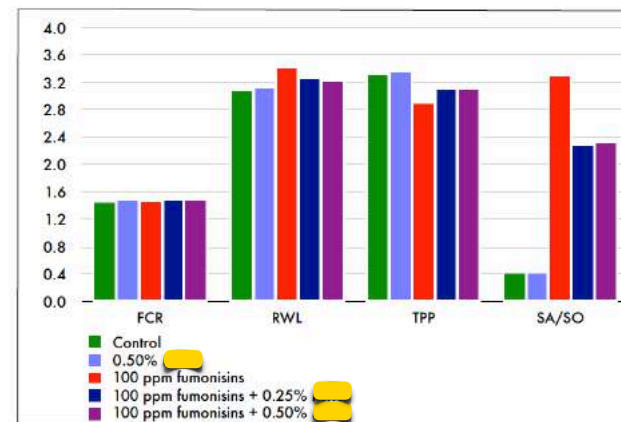


Figure 3 – Feed conversion rate (FCR), relative weight of the liver (RWL; %), serum levels of total plasma proteins (TPP; g/dL), and Sphinganine/Sphingosine ratio (SA/SO) of the broilers fed with a diet containing fumonisin, supplemented or not with [], for 21 days

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CONCLUSION

1. The deleterious effects of a diet containing 100 ppm of fumonisins on the broilers chicken evaluated parameters were showed during the experimental period of 21 days (P<0.05);
2. According to the evaluated parameters, 0.25% and 0.50% of [redacted] decreased the deleterious effects caused by 100 ppm of fumonisins added to the broilers chicken feed during the experimental period of 21 days (P<0.05).

The results obtained in the present experiment refer to the product available from OLMIX SA and are specific for the sent sample. Any future changes in the product, both in chemical or physical composition, must be subject to further evaluation.

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June 23rd, 2016
 Santa Maria/RS - Brazil

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 Director - Instituto SAMITEC

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 Professor at UFSM
 Scientific advisor - LAMIC/UFSM

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TECHNICAL REPORT: EFFECTIVENESS OF THE ADDITIVE ANTI MYCOTOXIN [redacted] IN DECREASE THE EFFECTS OF T-2 TOXIN ON CHICKENS

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Table 5 - Body weight of the broilers fed with a diet containing T-2 Toxin, supplemented or not with [redacted], for 21 days

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a - b = Averages in columns with different letters differ by the Bonferroni test (P<0.05).
¹BW = Body weight (g).
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Figure 3 - Feed conversion rate (FCR), relative weight of the liver (RWL; %), serum levels of total plasma proteins (TPP; g/dL), and Sphinganine/Sphingosine ratio (SA/SO) of the broilers fed with a diet containing fumonisins, supplemented or not with [redacted], for 21 days

Parameter	Control	0.50% [redacted]	100 ppm fumonisins	100 ppm fumonisins + 0.25% [redacted]	100 ppm fumonisins + 0.50% [redacted]
FCR	~1.4	~1.4	~1.4	~1.4	~1.4
RWL	~3.0	~3.0	~3.4	~3.2	~3.2
TPP	~3.3	~3.3	~2.8	~3.0	~3.0
SA/SO	~0.4	~0.4	~3.3	~2.2	~2.2

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ISO 9001:2015
 ISO 17025:2017

CONCLUSION

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- Corn (Aflatox) AAM RESULTS
- Corn (Fumonisins)
- LAMIC - Origin of Samples

LAMIC - Laboratory of Micotoxological Analysis

Complete the form below to access your Results - Anti Mycotoxins Additives (AAMI).

EXTRANET

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The Analytical Laboratory Mycotoxicology consumed by all Brazilians. In our country reliability and quality control LAMIC.

The Analytical Laboratory Mycotoxicology c...
 and guarantee the nutritional safety...

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Password: *

AAMI RESULTS

Aditivos Antimicotóxicos (AAM) avaliados (2005-2017)				
MICOTOXINAS	PRODUTOS AVALIADOS	% APROVADOS	IN VIVO DENTRO DO PRAZO*	IN VIVO - IN VÍTRIO DENTRO DO PRAZO*
 Aflatoxinas Fumonisinas Toxina T-2	95	48,9	8	2
	52	40,4	4	2
	5	20,0	1	0
 Aflatoxinas + Fumonisinas + Toxina T-2	22	36,3	8	0
 Aflatoxinas Aflatoxinas + Fumonisinas + Toxina T-2	1	100,0	1	1
	1	100,0	1	1
 Aflatoxinas Aflatoxinas	1	100,0	0	0
	4	100,0	0	0
 Aflatoxinas Fumonisinas Aflatoxinas	3	66,7	0	0
	2	50,0	1	0
	16	50,0	3	1
 Fumonisinas Zearalenona	20	42,5	1	1
	76	20,0	2	0
 Toxina T-2 Aflatoxinas + Fumonisinas + Toxina T-2	1	0,0	0	0
	12	0,0	0	0
Aflatoxina M1 Total	4	50,0	0	0
	315	36,3	30	8

* De acordo com a Portaria nº 13 de 24 de Maio de 2006 do Ministério da Agricultura, Pecuária e Abastecimento (MAPA)

CONCLUDING

