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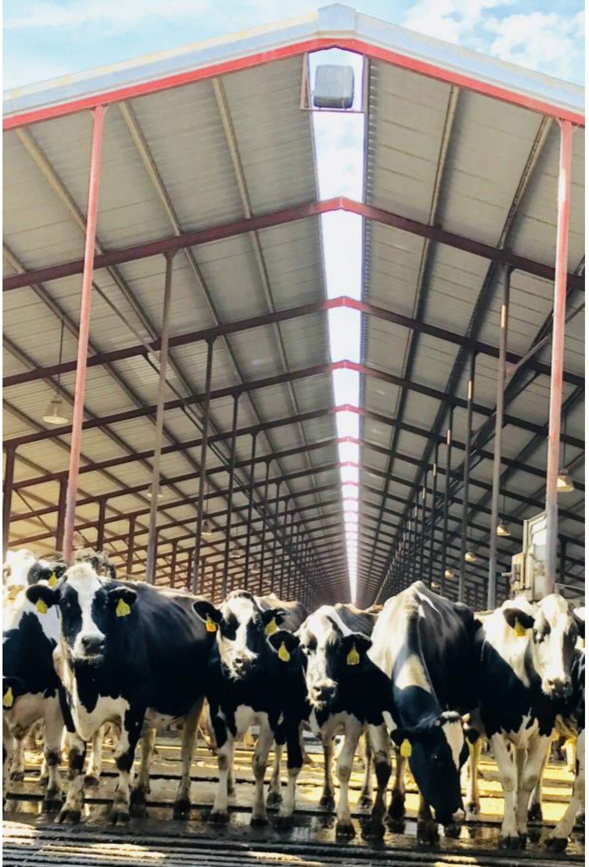

MITIGATION STRATEGIES IMPROVE PERFORMANCE AND REDUCE INFLAMMATION IN AFLATOXIN CHALLENGED DAIRY COWS

Russell T. Pate* and Felipe C. Cardoso

Monday August 26th, 2019

EAAP 2019 Annual Meeting, Ghent, Belgium

Abstract #31027

Outline

Introduction to aflatoxins

- Stress
- Mitigation strategies

Evaluation of mitigation strategies

- Injectable trace minerals
- Aluminosilicate clay

Conclusions

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Aflatoxins

Aflatoxin B_1 (AFB1)

Aspergillus flavus and A. parasiticus

Transformed to aflatoxin M_1 (AFM1)

AFB1 and AFM1 are carcinogenic

20 ppb and 0.5 ppb in US

5 ppb and 0.05 ppb in EU

Up to $1.68 billion contamination loss

Negative impact on dairy cattle health

Inappetence, lethargy, reproductive disorders

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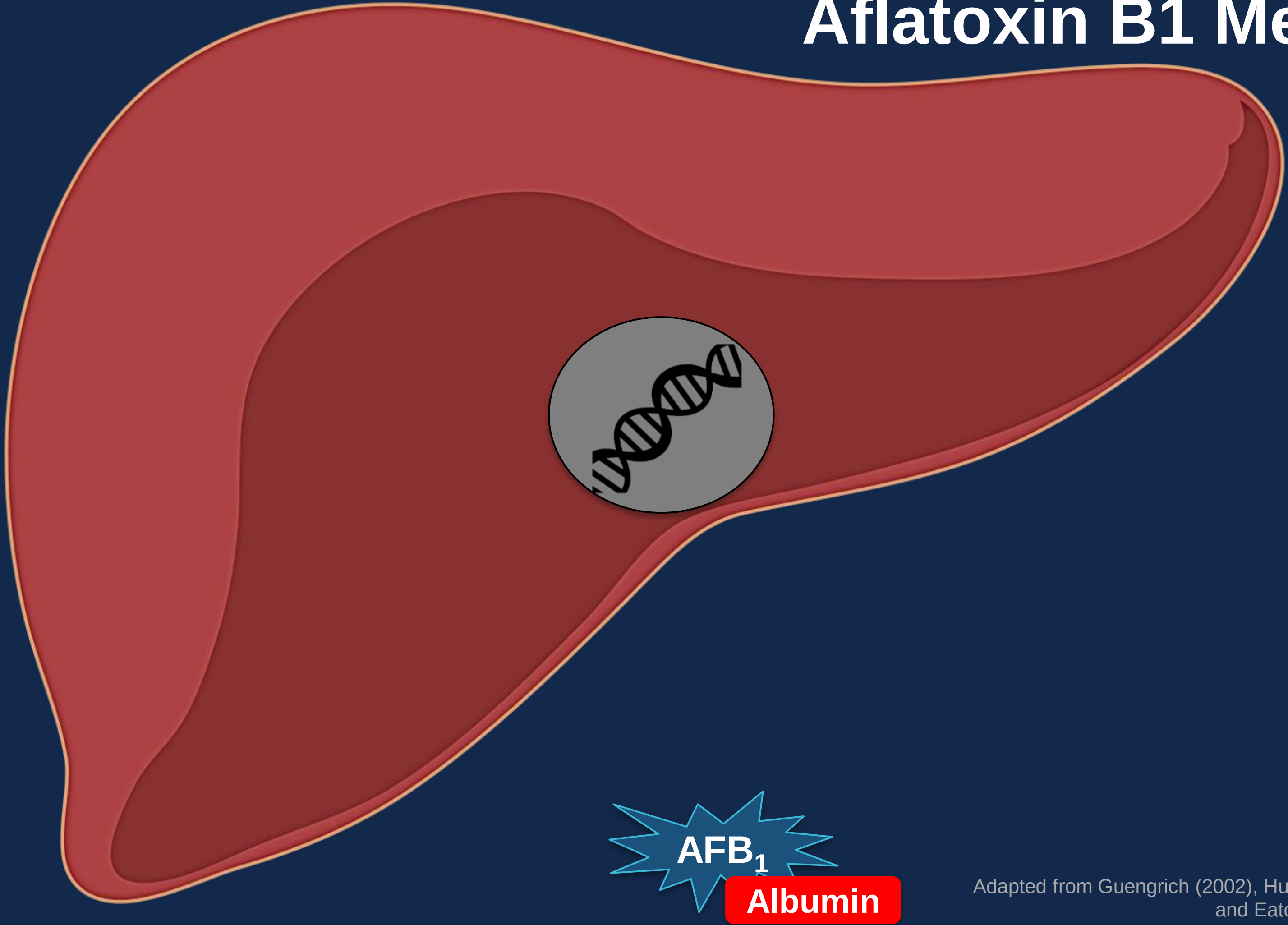
Dairy Focus Lab

FDA (1994), Liu and Wu (2010), and Mitchell et al. (2016)

Aflatoxin B1 Metabolism



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Dairy Focus Lab

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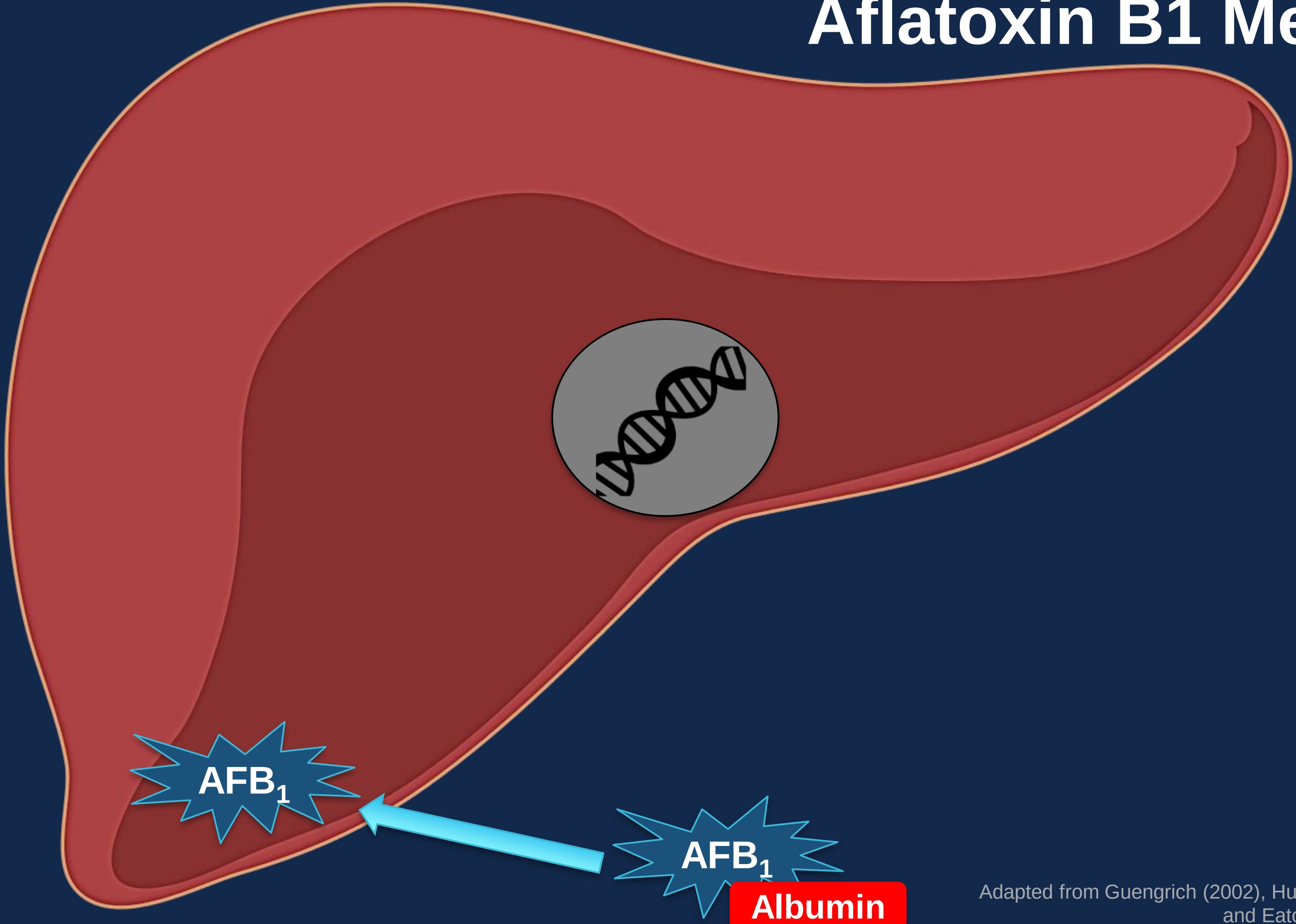

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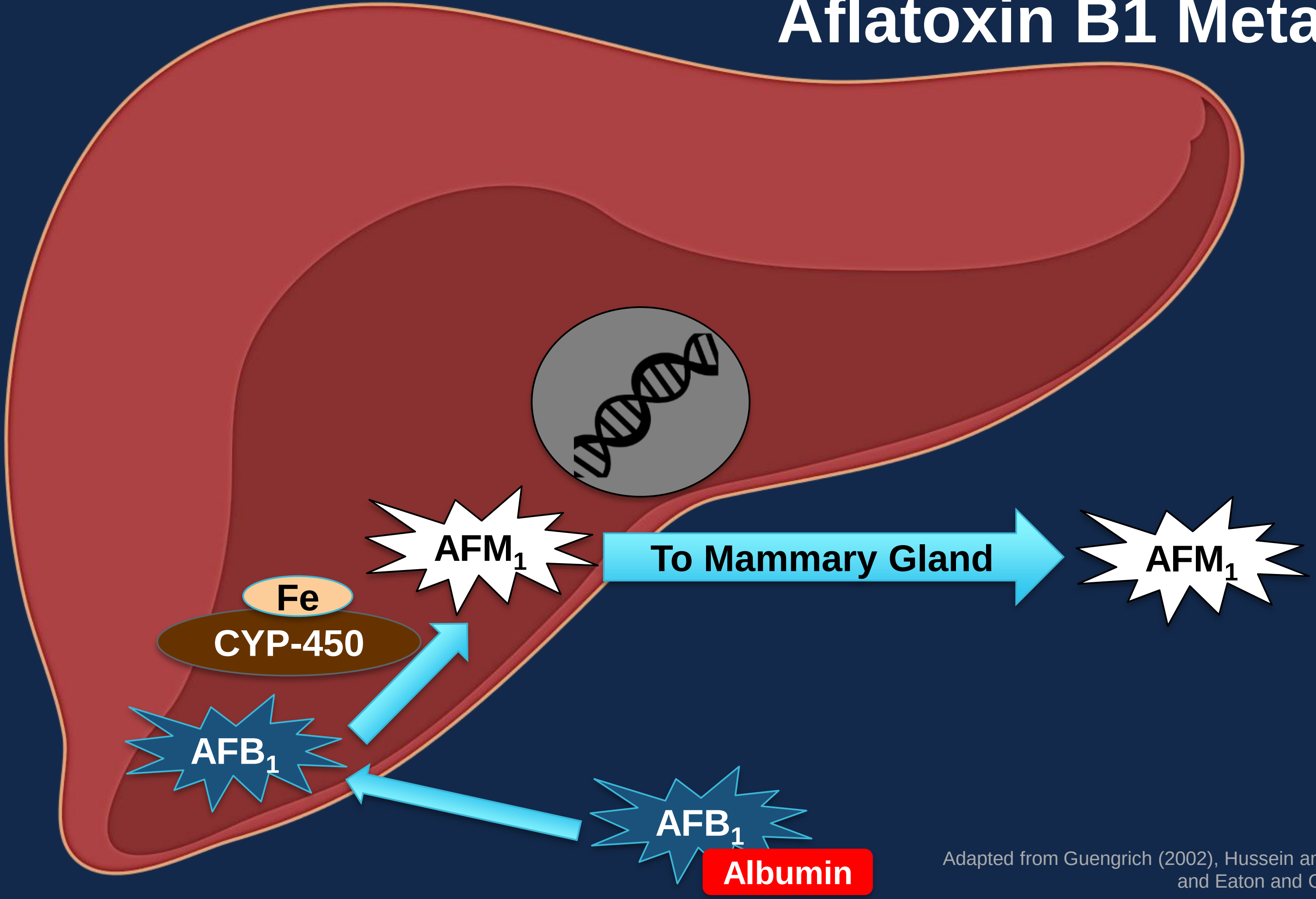

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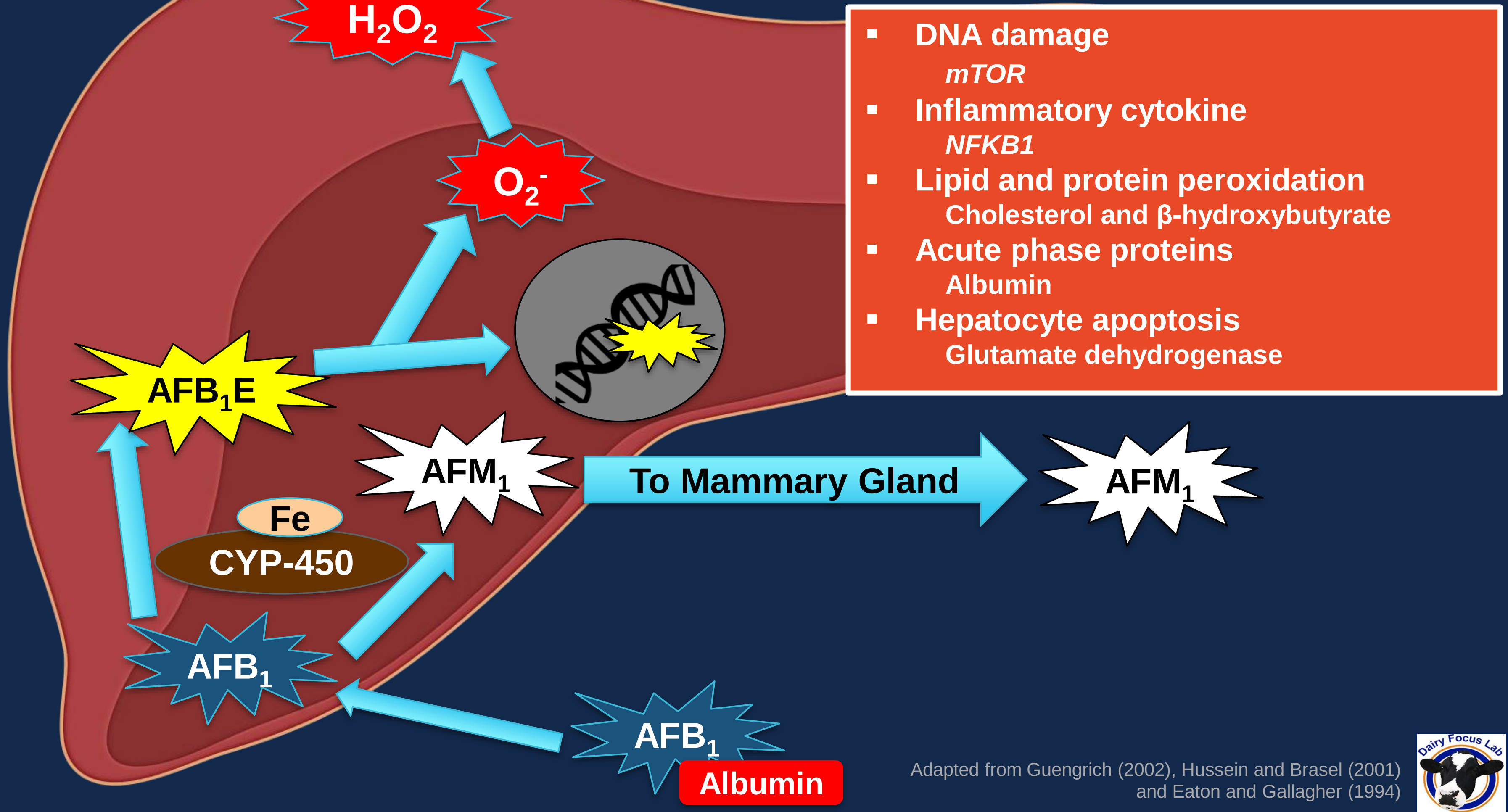

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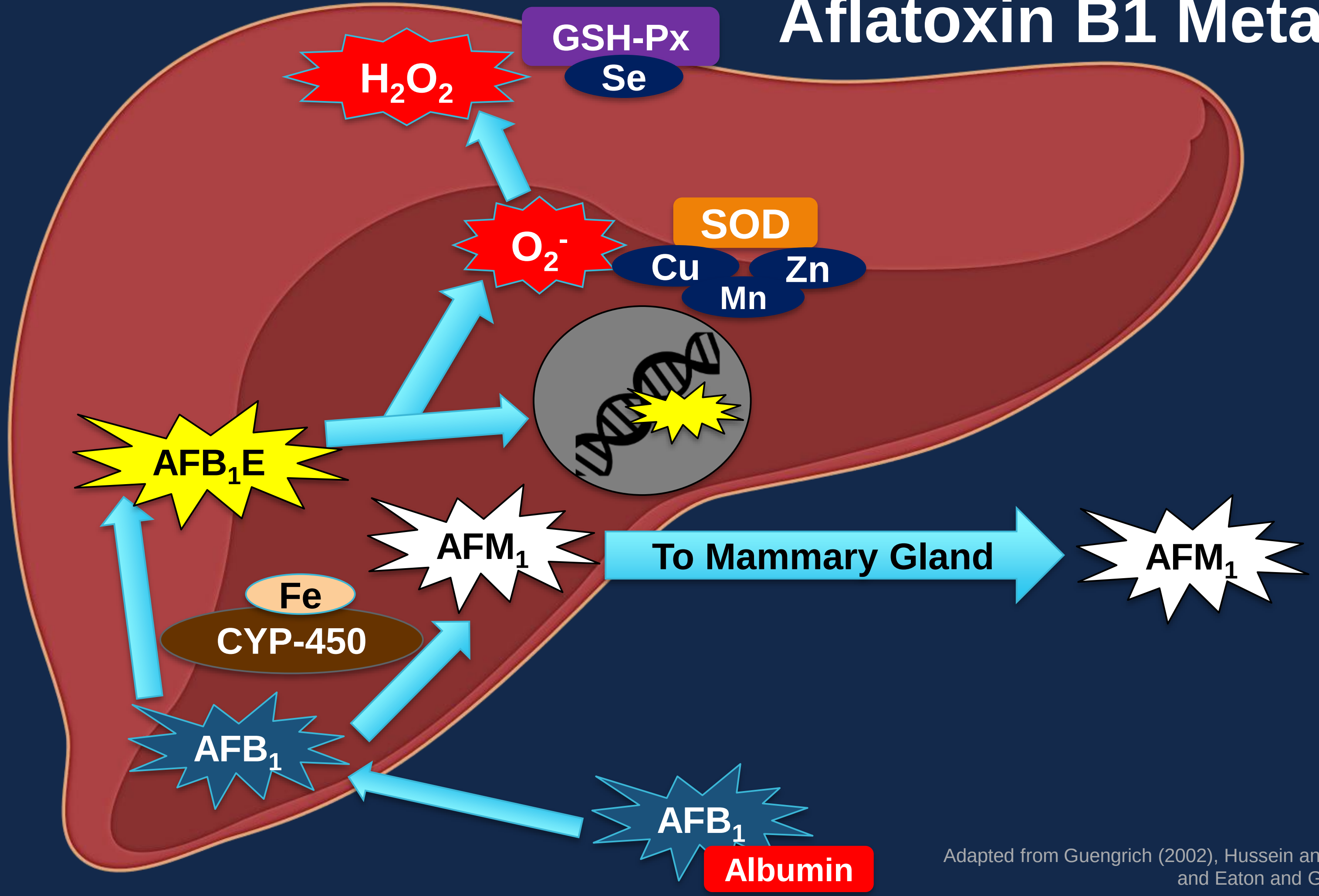

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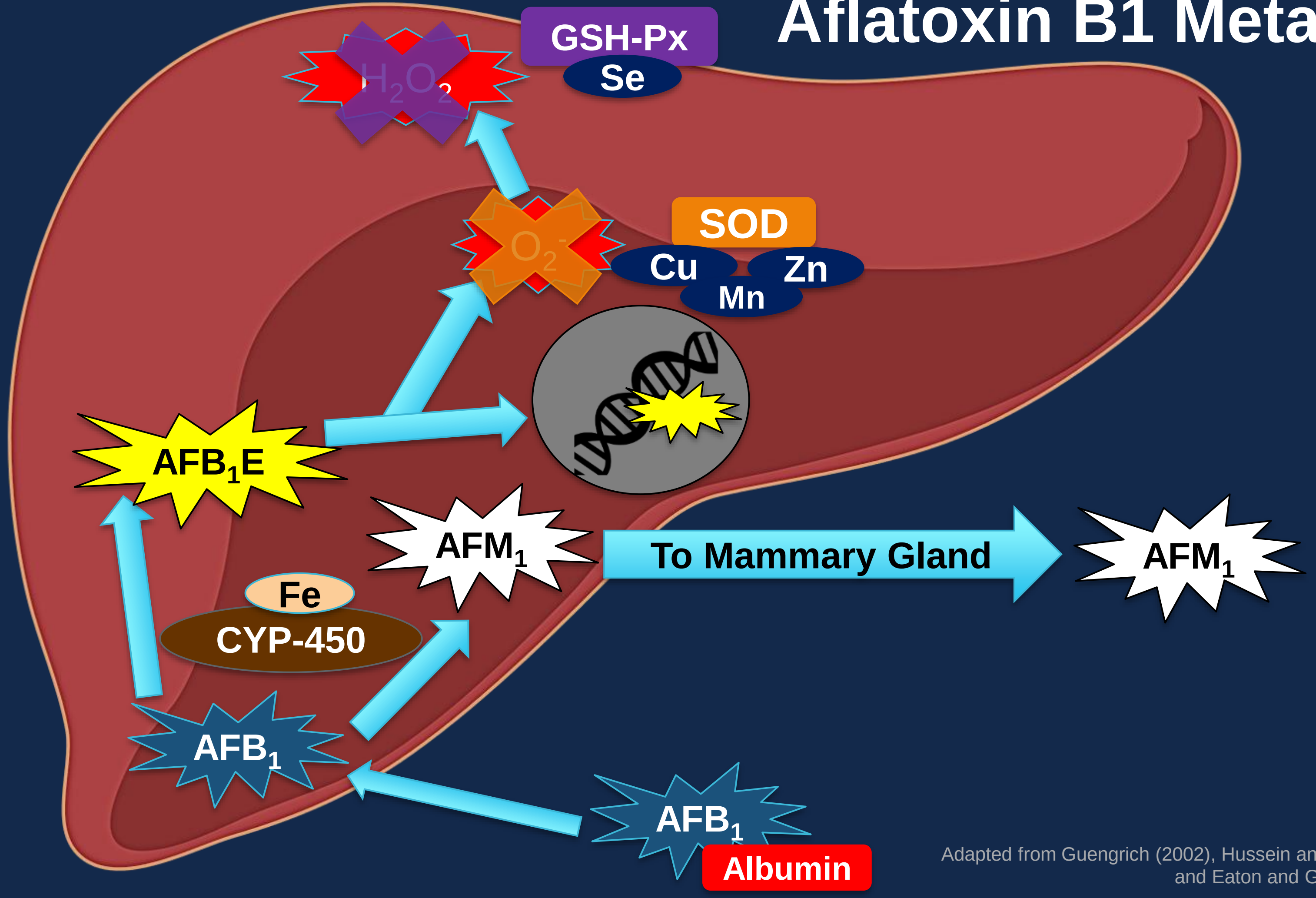

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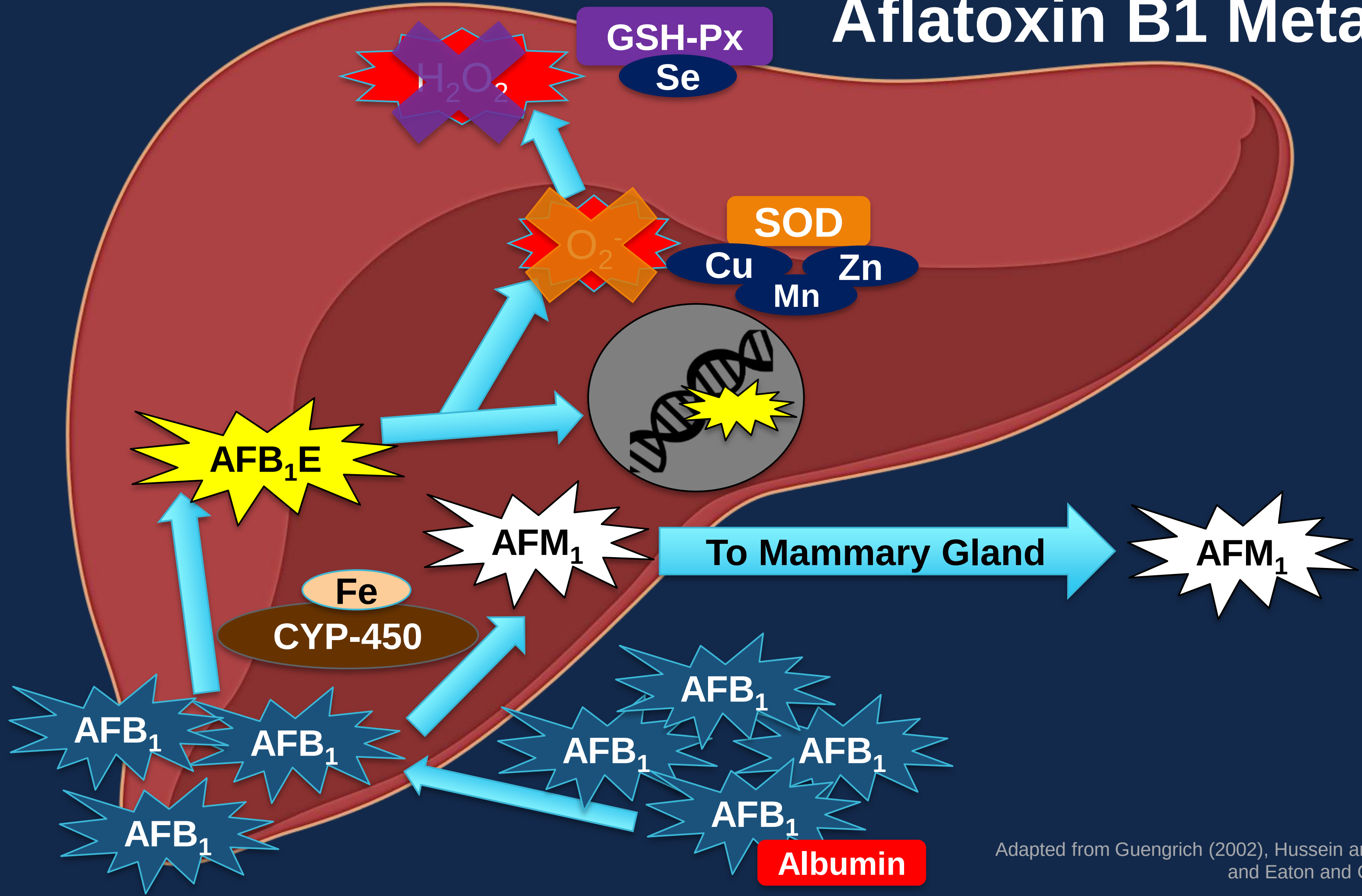

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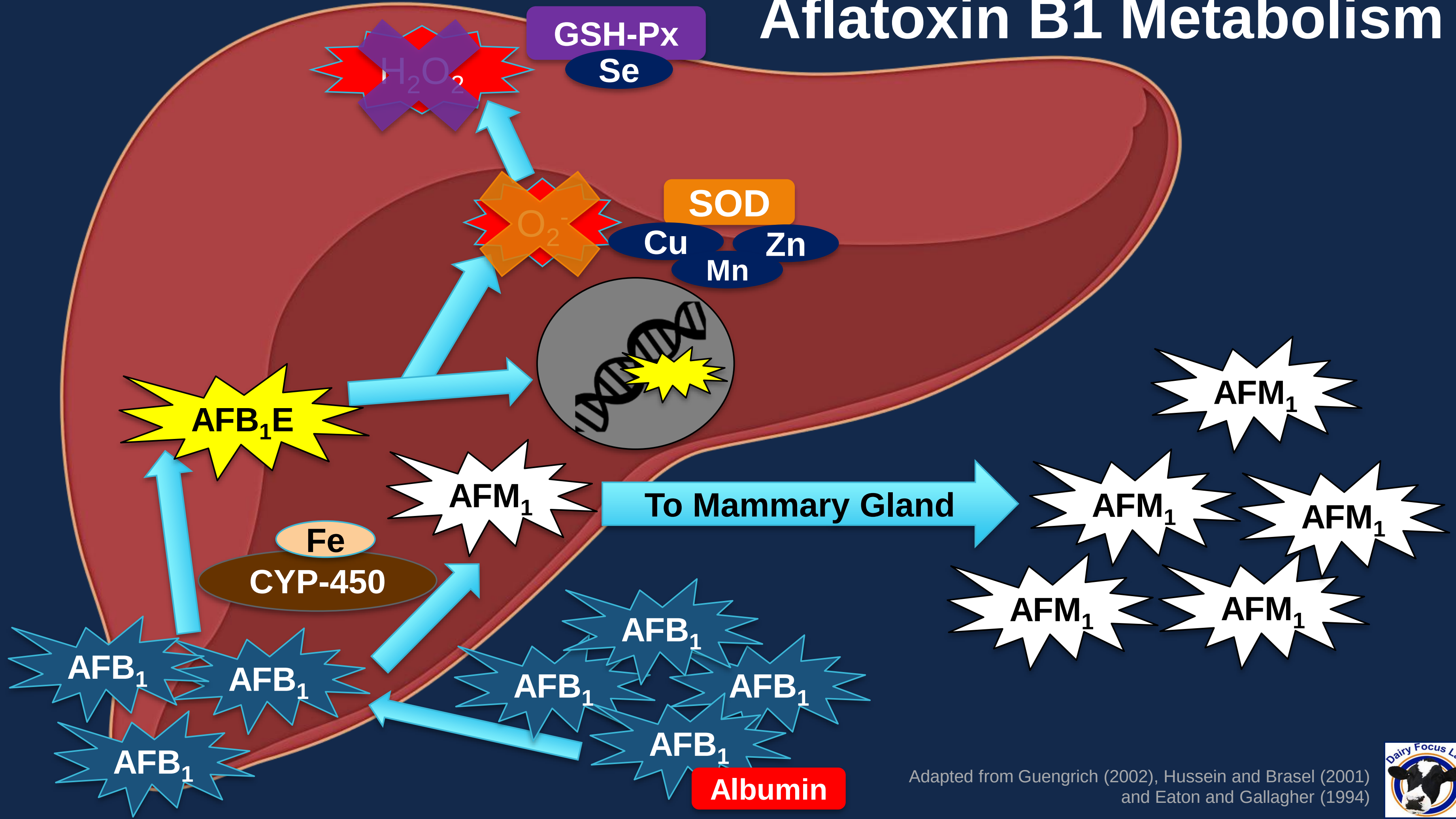

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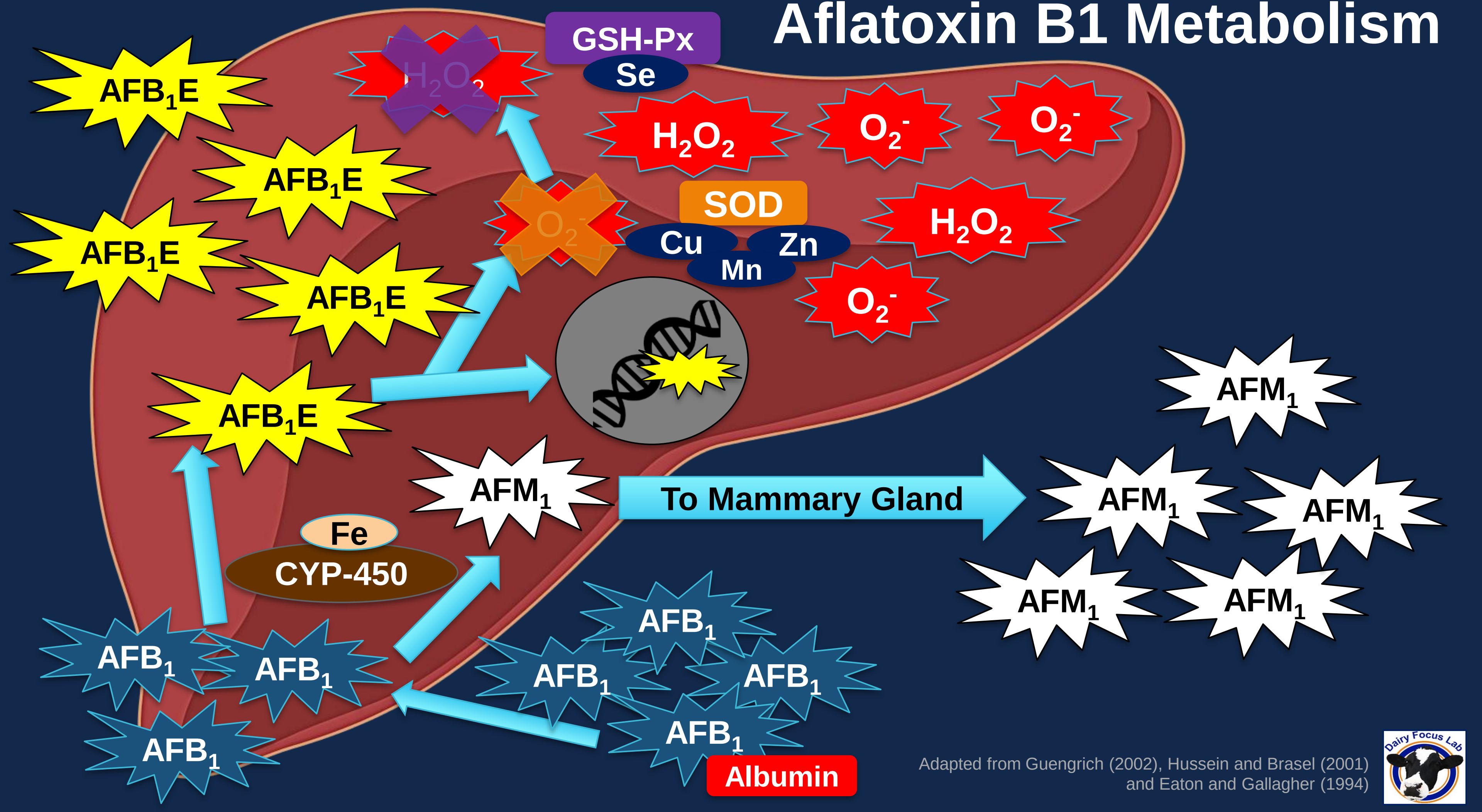

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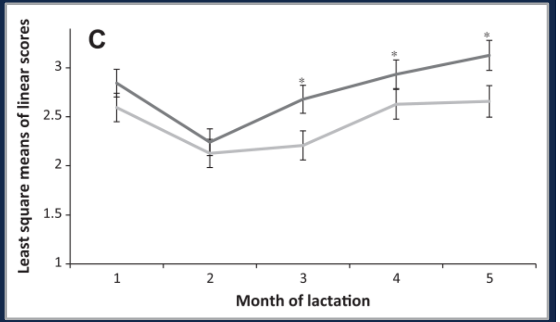
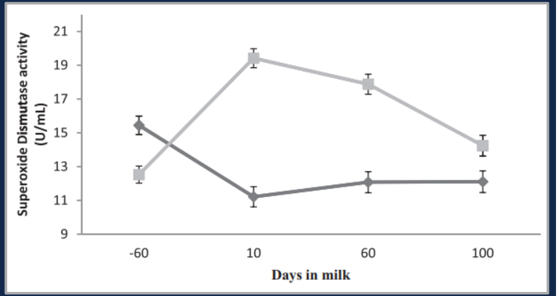
Trace Mineral Supplementation





Needed in relatively small amounts (ppm or ppb)

- Cu, Zn, Mn, Se, and Fe

Important for immune function and antioxidant activity

- Cytochrome P450, SOD and GSH-Px

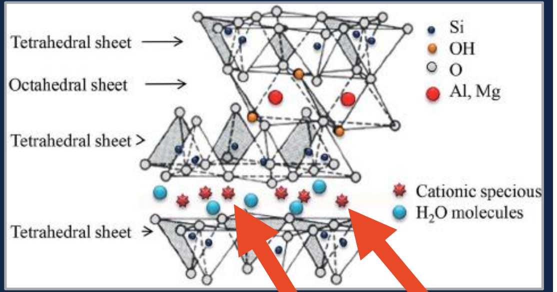
Trace mineral status variable

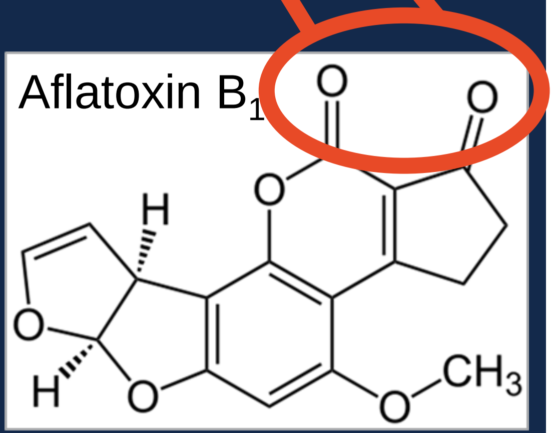
- Intake dependent
- Physiological status
- Interaction with diet constituents

Injectable trace mineral allows for consistent status

Limited research between trace mineral and AF exposure

Sordillo (2003), Herdt and Hoff (2011), Machado et al. (2013), and Teixeira et al. (2014)





Sequestering agents

Clay-based, yeast cell wall, activated charcoals

Limit AF bioavailability via ion exchange

Reduce AF concentrations in milk, urine, and feces

May improve immune response to aflatoxin

- Neutrophil migration

More research needed to elucidate the effects of mitigation strategies during an AF challenge on oxidative stress

Moschini et al. (2008), Ghadari et al. (2015), Ogunade et al. (2016), and Sulzberger et al. (2017)

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J. Dairy Sci. 101:8532–8543
https://doi.org/10.3168/jds.2018-14447

Injectable trace minerals (selenium, copper, zinc, and manganese) alleviate inflammation and oxidative stress during an aflatoxin challenge in lactating multiparous Holstein cows

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Department of Animal Sciences, University of Illinois, Urbana 61801

ABSTRACT

Trace minerals are vital in the antioxidant response during oxidative stress; however, limited research is available on the effects of trace mineral supplementation during an aflatoxin (AF) challenge. The objective of the study was to determine the effects of 2 subcutaneous injections of 15 mg/mL of Cu, 5 mg/mL of Se, 60 mg/mL of Zn, and 10 mg/mL of Mn (Multimin 90, Multimin North America, Fort Collins, CO) given at 1 mL/90.7 kg of average body weight in response to an AF challenge. Fifty-eight Holstein cows [body weight (mean ± SD) = 734 ± 6 0kg; days in milk = 191 ± 93] were assigned to 1 of 3 treatments in a randomized complete block design. The experimental period (63 d) was divided into an adaptation phase (d 1–56) and a measurement phase (d 57–63). From d 57 to 59, cows received an AF challenge that consisted of 100 µg of aflatoxin B_1/kg of dietary dry matter intake (DMI) administered orally via balling gun. Treatments were saline injection and no AF challenge (NEG), saline injection and AF challenge (POS), and trace mineral injection and AF challenge (MM). Injections were performed subcutaneously on d 1 and 29. Milk was sampled 3 times daily from d 56 to 63, blood was sampled on d 0, 56, 60, and 63, and liver samples were taken on d 0 and 60. Two treatment orthogonal contrasts [CONT1 (NEG vs. POS) and CONT2 (POS vs. MM)] were made. Cows in NEG had lower AF excretion in milk and greater 3.5% fat-corrected milk (32.1 ± 1.37 kg/d) compared with cows in POS (28.6 ± 1.43 kg/d). Feed efficiencies (3.5% fat-corrected milk/DMI, energy-corrected milk/DMI, and milk/DMI) were greater for cows in NEG (1.42 ± 0.07, 1.46 ± 0.07, and 1.45 ± 0.07, respectively) than cows in POS (1.16 ± 0.08, 1.18 ± 0.08, and 1.22 ± 0.07, respectively). Cows in POS had greater milk urea nitrogen and blood urea nitrogen than cows in MM. Liver concentrations of Se and Fe were greater for cows in MM compared with cows in POS. Cows in MM tended to have greater plasma glutathione peroxidase activity compared with cows in POS. An upregulation of liver *GPX1* was observed for cows in POS compared with cows in MM. In conclusion, subcutaneous injection of trace minerals maintained an adequate antioxidant response when an AF challenge was present.
Key words: aflatoxin, liver, trace minerals, AFM_1

INTRODUCTION

An estimated $0.11 to $1.68 billion is lost annually due to the effects of mycotoxins on corn crops (Mitchell et al., 2016). Mycotoxins are toxins produced by fungi growing on feed crops such as corn, with the 3 most common being aflatoxin (**AF**), fumonisin, and deoxynivalenol (Flores-Flores et al., 2015; Mitchell et al., 2016). Aflatoxin B_1 (**AFB_1**), an aflatoxin derivative produced by *Aspergillus parasiticus* and *flavus*, is hydroxylated and demethylated in the liver to aflatoxin M_1 (**AFM_1**) after ingestion (Kuilman et al., 2000). Aflatoxin B_1 and AFM_1 are classified as group 1 carcinogens by the International Agency for Research on Cancer (Liu and Wu, 2010); therefore, the FDA has set limits on AF concentration in feedstuffs and milk to be 20 and 0.5 µg/kg, respectively (Peraica et al., 1999).

Aflatoxin exposure causes adverse effects in dairy cattle, such as inappetence, immunosuppression, decreased milk production, and reproductive disorders (Abrar et al., 2013; Sulzberger et al., 2017). Aflatoxin B1 is believed to increase oxidative stress through the production of reactive oxidative species, primarily superoxide anions and hydrogen peroxides in the liver (Guengerich et al., 2001). Superoxide dismutase (**SOD**) is a Zn-, Mn-, and Cu-dependent enzyme linked to oxidative stress and the reduction of reactive oxidative species (Machado et al., 2014). Weatherly et al. (2018) observed greater plasma SOD concentrations for cows challenged with AF (2.77 U/mL) than cows not challenged (1.96 U/mL). Additionally, reports show that AFB_1 increases bovine peripheral blood mononuclear

Received January 15, 2018.
Accepted May 3, 2018.
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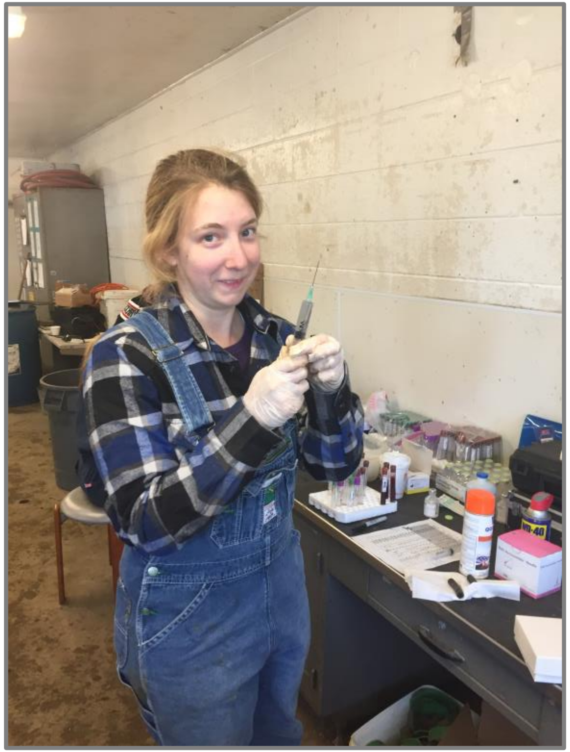
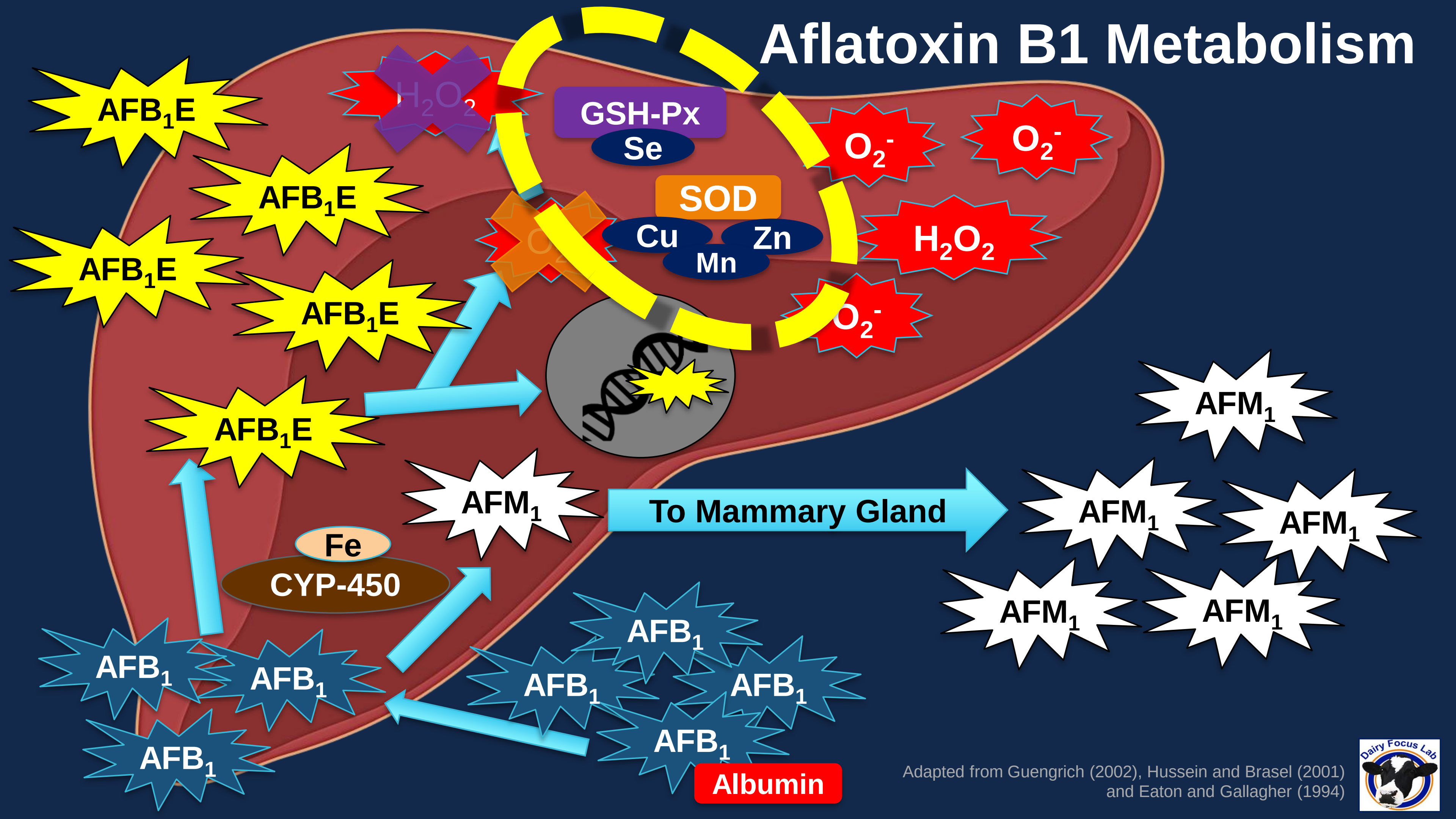
Experimental Hypothesis

Cows receiving mineral injection (Cu, Zn, Mn, and Se) would experience lower oxidative stress due to an AF challenge than cows not receiving the mineral injection




Aflatoxin B1 Metabolism
AFB_1E
AFB_1E
AFB_1E
AFB_1E
AFB_1E
H_2O_2
GSH-Px
Se
SOD
Cu
Zn
Mn
O_2^-
O_2^-
H_2O_2
O_2^-
AFM_1
To Mammary Gland
AFM_1
AFM_1
AFM_1
AFM_1
AFM_1
Fe
CYP-450
AFB_1
AFB_1
AFB_1
AFB_1
AFB_1
AFB_1
AFB_1
Albumin
Adapted from Guengrich (2002), Hussein and Brasel (2001) and Eaton and Gallagher (1994)
Dairy Focus Lab

Materials and Methods

Randomized complete block design

- 21 blocks
- 63 days (Adaption: 1-56 | Measurement: 57-63)

58 multiparous Holstein cows

- 168 ± 95 d in milk

3 treatments

- **NEG**- Placebo (saline) injection + no AF challenge
- **POS**- Placebo (saline) injection + AF challenge
- **MM**- Trace mineral injection + AF challenge

100 µg of AFB1 / kg of DMI

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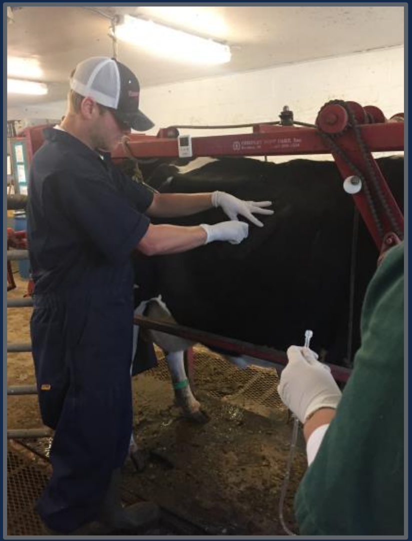
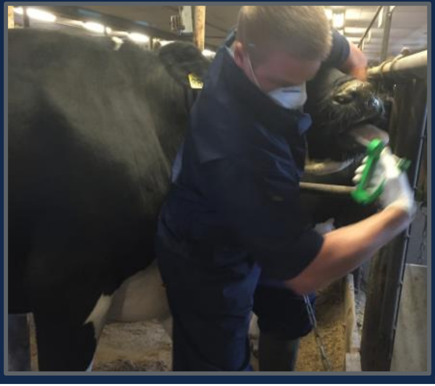




Experimental Timeline

- = Blood
- = Liver
- = Trace mineral injection
- = Aflatoxin challenge

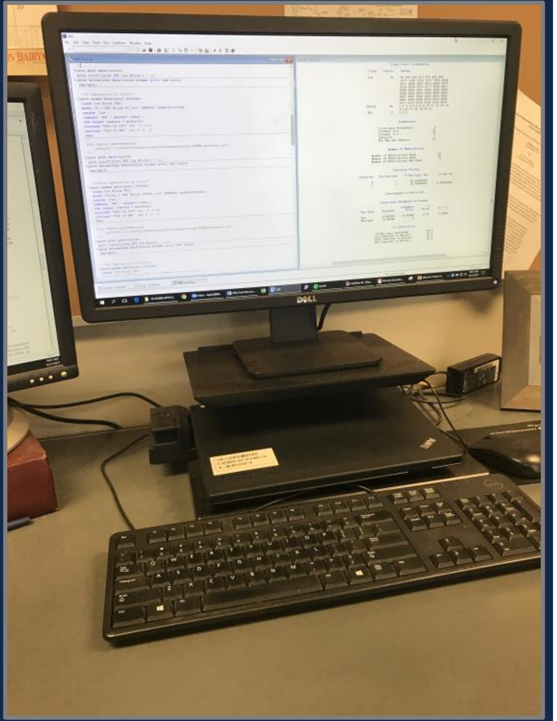
Daily

Milk (3x)
Dry Matter Intake
Health checks

Weekly

Body Weight
BCS

Statistical Analysis



SAS (v. 9.4, SAS Institute Inc., Cary, NC)
- MIXED Procedure

2 orthogonal contrasts
- POS vs. NEG
- POS vs. MM

Significance declared at $P \leq 0.05$

Trends declared at $0.05 < P \leq 0.10$

Ingredient	% of DM
Corn silage	36.37
Canola meal	11.71
Alfalfa hay	11.20
Corn gluten feed	8.29
Soy hulls	4.29
Wheat straw	2.34
Dry ground corn grain	19.25
Concentrate mix	6.56

*100 µg of AFB1 / kg of DMI (i.e. 100 ppb)

Item	Mean	SD	NRC
DM, %	46.6	1.24	
CP	17.5	0.39	
ADF	18.4	1.29	
NDF	30.6	1.16	
Starch	29.1	1.93	
Crude fat	3.9	0.73	
Ash	9.96	0.10	
Fe, ppm	468.7	44.91	
Zn, ppm	97.3	6.94	63.0
Cu, ppm	18.00	0.82	15.7
Mn, ppm	99.0	7.35	16.7
Se, ppm	0.54	0.02	0.35

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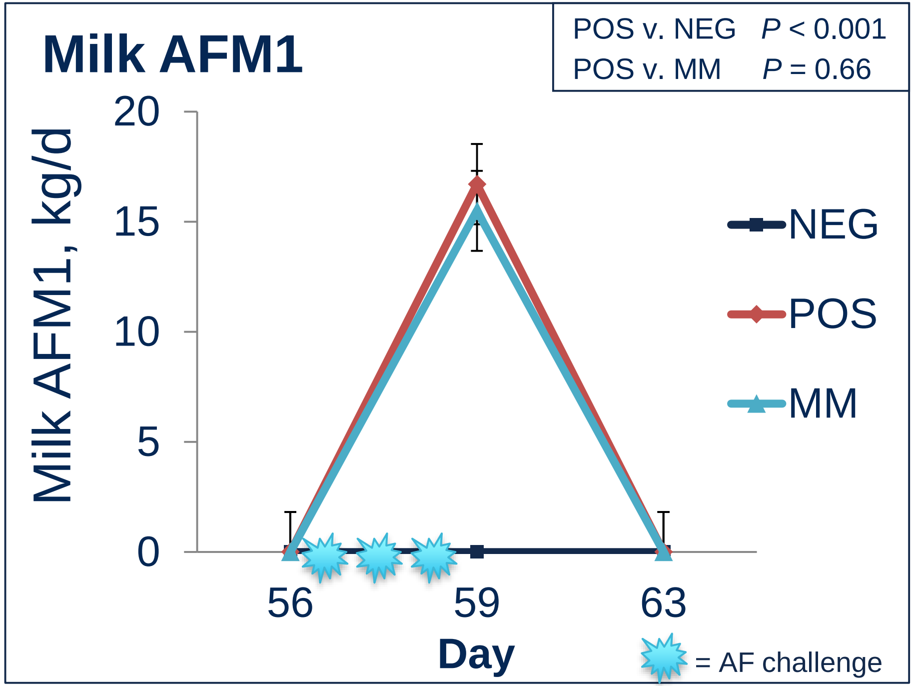
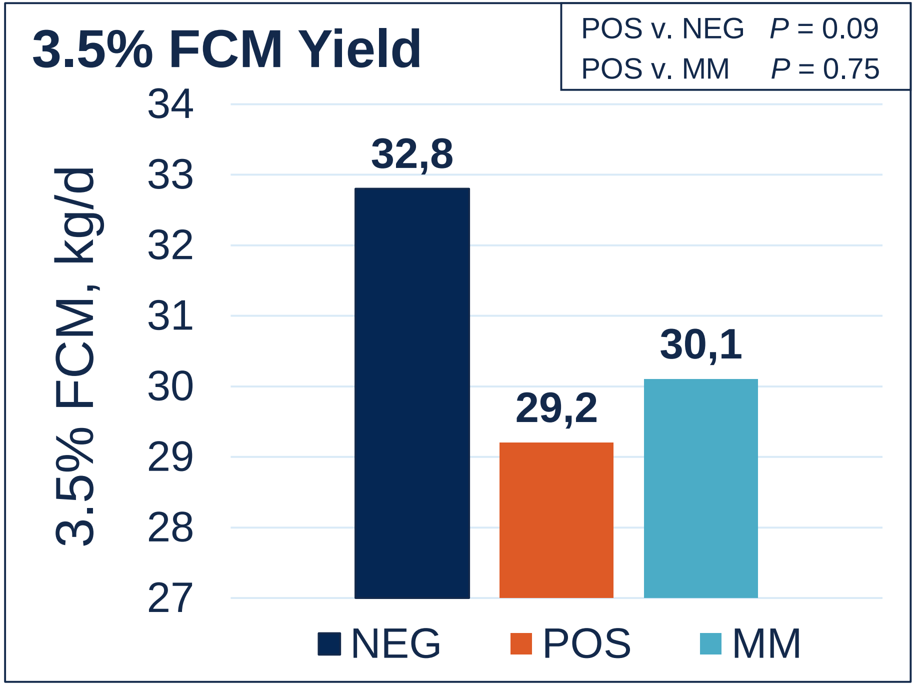
Cu, ppm	18.00	0.82	15.7
Mn, ppm	99.0	7.35	16.7
Se, ppm	0.54	0.02	0.35

Trace mineral injection neither hindered nor improved milk AFM1 excretion; AF challenge reduced 3.5% FCM





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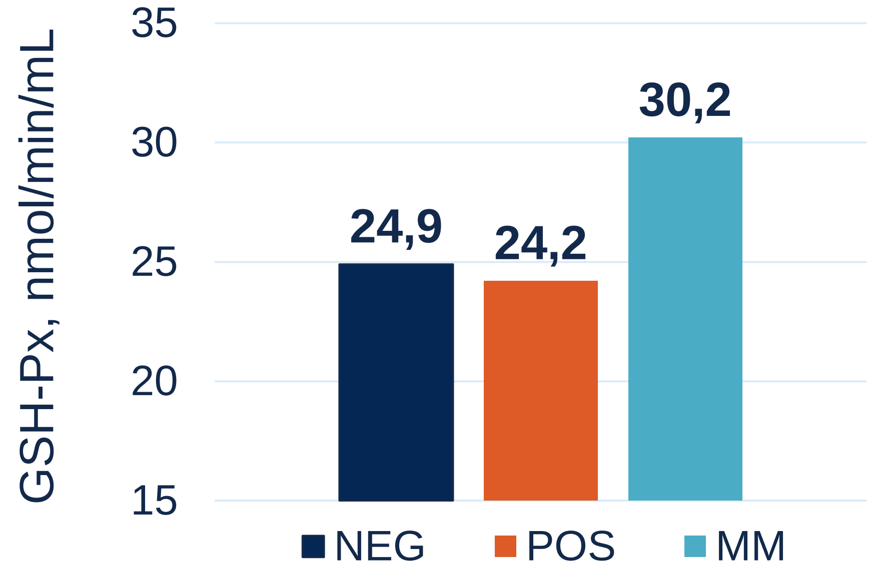
Pate and Cardoso (2018)

Trace mineral injection increased plasma GSH-Px activity and neither hindered nor improved plasma SOD

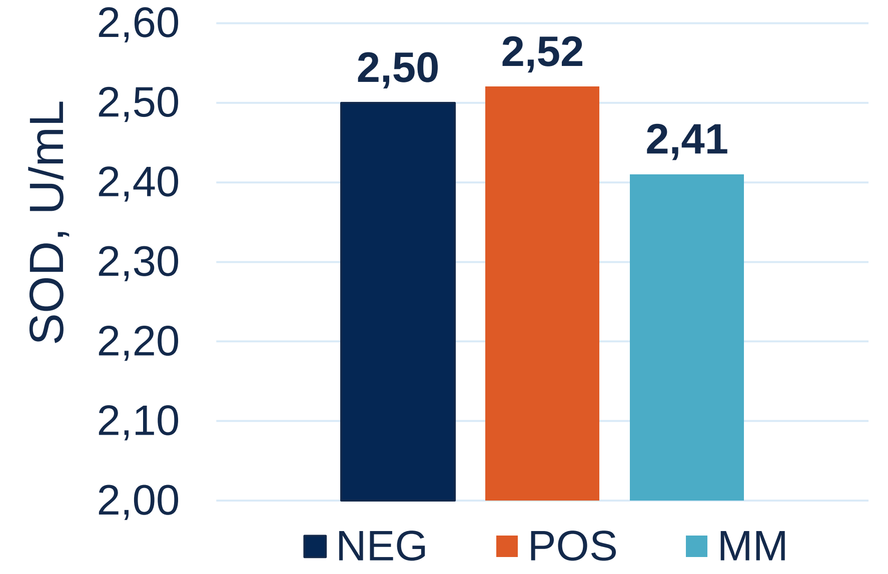
Glutathione peroxidase

POS v. NEG *P* = 0.86
POS v. MM *P* = 0.10

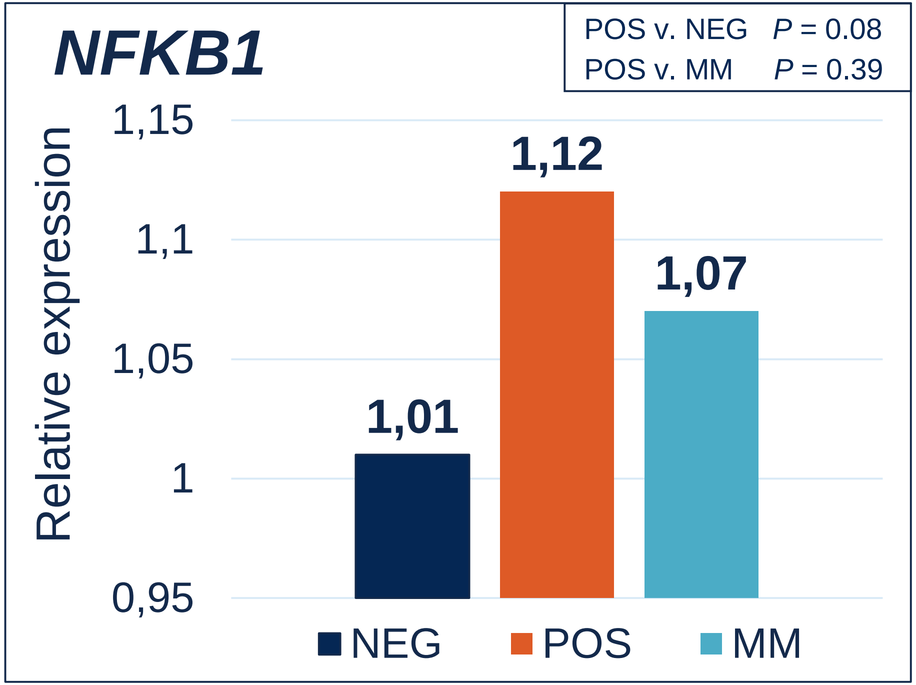


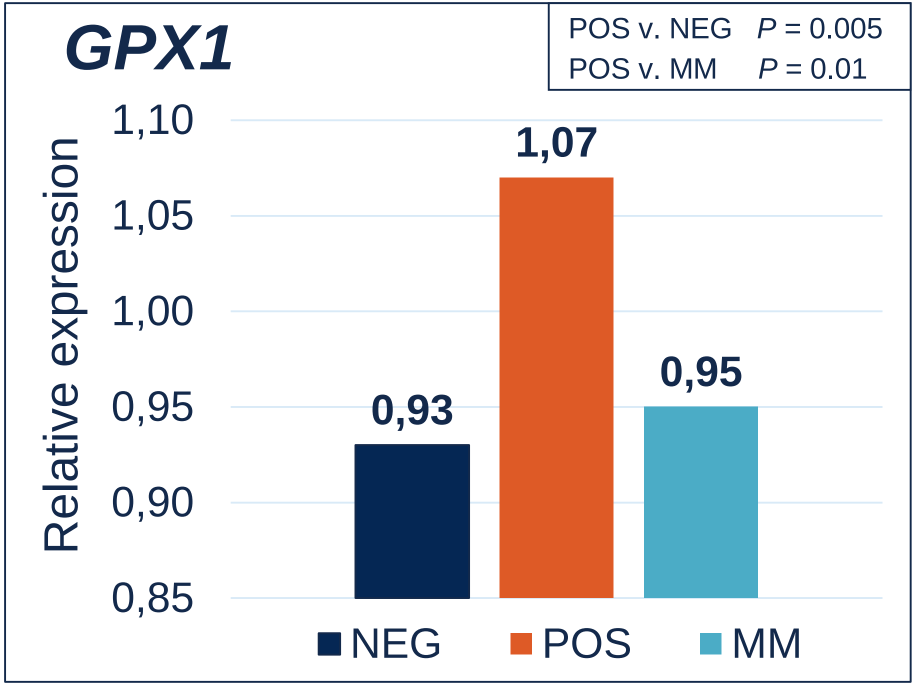
Superoxide dismutase

POS v. NEG *P* = 0.92
POS v. MM *P* = 0.48



AF challenge increased *NFKB1 and GPX1* expression; Trace mineral injection reduced *GPX1* expression





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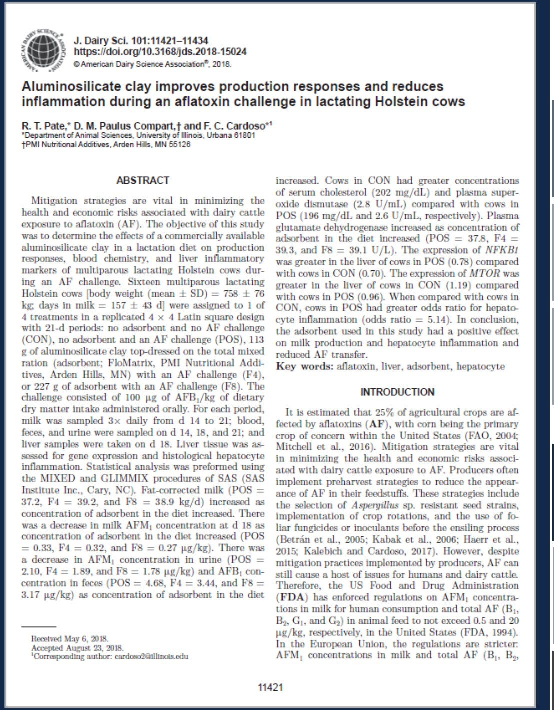

J. Dairy Sci. 101:11421–11434
https://doi.org/10.3168/jds.2018-15024

Aluminosilicate clay improves production responses and reduces inflammation during an aflatoxin challenge in lactating Holstein cows

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ABSTRACT

Mitigation strategies are vital in minimizing the health and economic risks associated with dairy cattle exposure to aflatoxin (AF). The objective of this study was to determine the effects of a commercially available aluminosilicate clay in a lactation diet on production responses, blood chemistry, and liver inflammatory markers of multiparous lactating Holstein cows during an AF challenge. Sixteen multiparous lactating Holstein cows [body weight (mean ± SD) = 758 ± 76 kg; days in milk = 157 ± 43 d] were assigned to 1 of 4 treatments in a replicated 4 × 4 Latin square design with 21-d periods: no adsorbent and no AF challenge (CON), no adsorbent and an AF challenge (POS), 113 g of aluminosilicate clay top-dressed on the total mixed ration (adsorbent; FloMatrix, PMI Nutritional Additives, Arden Hills, MN) with an AF challenge (F4), or 227 g of adsorbent with an AF challenge (F8). The challenge consisted of 100 μg of AFB_1/kg of dietary dry matter intake administered orally. For each period, milk was sampled 3× daily from d 14 to 21; blood, feces, and urine were sampled on d 14, 18, and 21; and liver samples were taken on d 18. Liver tissue was assessed for gene expression and histological hepatocyte inflammation. Statistical analysis was preformed using the MIXED and GLIMMIX procedures of SAS (SAS Institute Inc., Cary, NC). Fat-corrected milk (POS = 37.2, F4 = 39.2, and F8 = 38.9 kg/d) increased as concentration of adsorbent in the diet increased. There was a decrease in milk AFM_1 concentration at d 18 as concentration of adsorbent in the diet increased (POS = 0.33, F4 = 0.32, and F8 = 0.27 μg/kg). There was a decrease in AFM_1 concentration in urine (POS = 2.10, F4 = 1.89, and F8 = 1.78 μg/kg) and AFB_1 concentration in feces (POS = 4.68, F4 = 3.44, and F8 = 3.17 μg/kg) as concentration of adsorbent in the diet increased. Cows in CON had greater concentrations of serum cholesterol (202 mg/dL) and plasma superoxide dismutase (2.8 U/mL) compared with cows in POS (196 mg/dL and 2.6 U/mL, respectively). Plasma glutamate dehydrogenase increased as concentration of adsorbent in the diet increased (POS = 37.8, F4 = 39.3, and F8 = 39.1 U/L). The expression of *NFKB1* was greater in the liver of cows in POS (0.78) compared with cows in CON (0.70). The expression of *MTOR* was greater in the liver of cows in CON (1.19) compared with cows in POS (0.96). When compared with cows in CON, cows in POS had greater odds ratio for hepatocyte inflammation (odds ratio = 5.14). In conclusion, the adsorbent used in this study had a positive effect on milk production and hepatocyte inflammation and reduced AF transfer.
Key words: aflatoxin, liver, adsorbent, hepatocyte

INTRODUCTION

It is estimated that 25% of agricultural crops are affected by aflatoxins (**AF**), with corn being the primary crop of concern within the United States (FAO, 2004; Mitchell et al., 2016). Mitigation strategies are vital in minimizing the health and economic risks associated with dairy cattle exposure to AF. Producers often implement preharvest strategies to reduce the appearance of AF in their feedstuffs. These strategies include the selection of *Aspergillus* sp. resistant seed strains, implementation of crop rotations, and the use of foliar fungicides or inoculants before the ensiling process (Betrán et al., 2005; Kabak et al., 2006; Haerr et al., 2015; Kalebich and Cardoso, 2017). However, despite mitigation practices implemented by producers, AF can still cause a host of issues for humans and dairy cattle. Therefore, the US Food and Drug Administration (**FDA**) has enforced regulations on AFM_1 concentrations in milk for human consumption and total AF (B_1, B_2, G_1, and G_2) in animal feed to not exceed 0.5 and 20 μg/kg, respectively, in the United States (FDA, 1994). In the European Union, the regulations are stricter: AFM_1 concentrations in milk and total AF (B_1, B_2,

Received May 6, 2018.
Accepted August 23, 2018.
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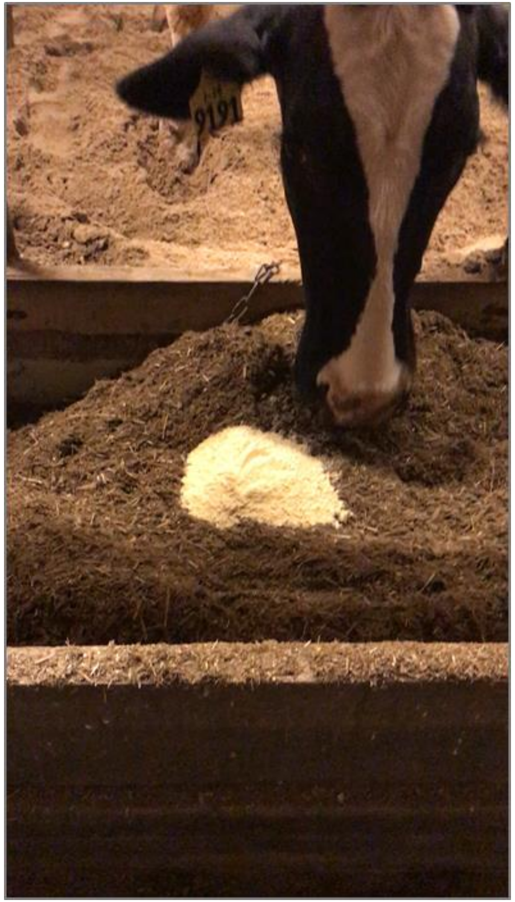
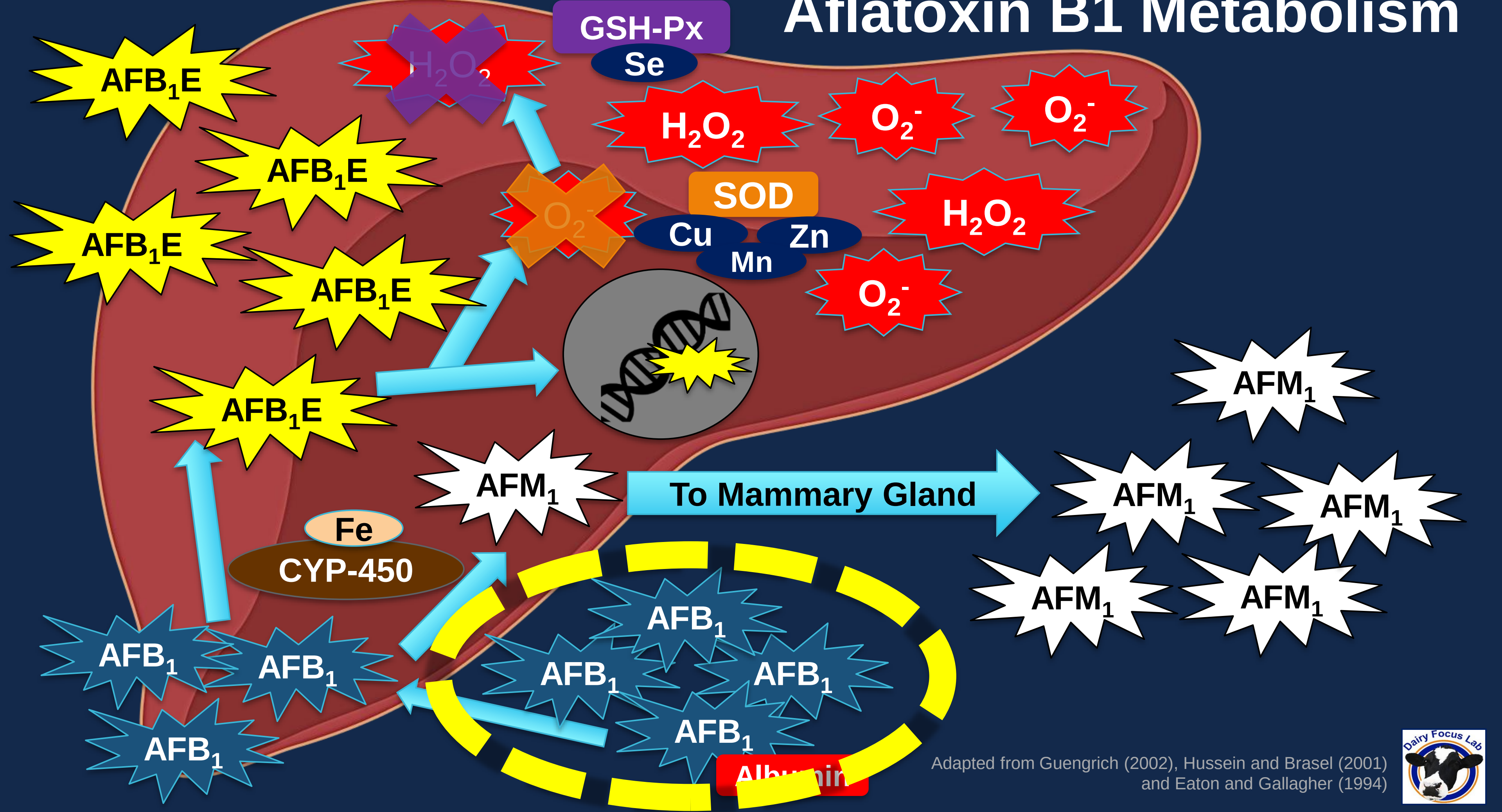
Experimental Hypothesis

Cows receiving aluminosilicate clay would experience lower AF excretion in milk and would therefore exhibit lower oxidative stress than cows not receiving adsorbent



Aflatoxin B1 Metabolism



Adapted from Guengrich (2002), Hussein and Brasel (2001) and Eaton and Gallagher (1994)

Materials and Methods

Replicated 4 × 4 Latin square design

21 day periods

16 multiparous Holstein cows

157 ± 43 days in milk

4 treatments

CON- 300 g corn grain + **<u>no</u>** AF challenge
POS- 300 g corn grain + AF challenge
F4- 113 g (4 oz.) aluminosilicate clay* + AF challenge
F8- 226 g (8 oz.) aluminosilicate clay* + AF challenge

100 µg of AFB1 / kg of DMI

* Mixed with 300 g corn grain

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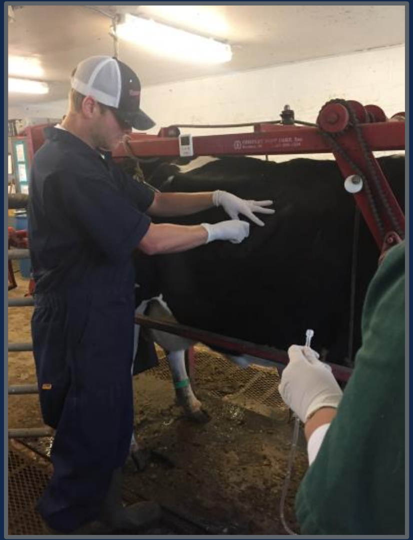
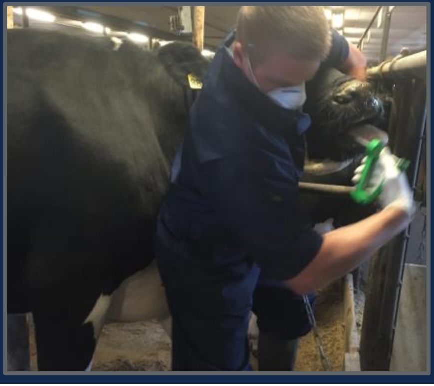
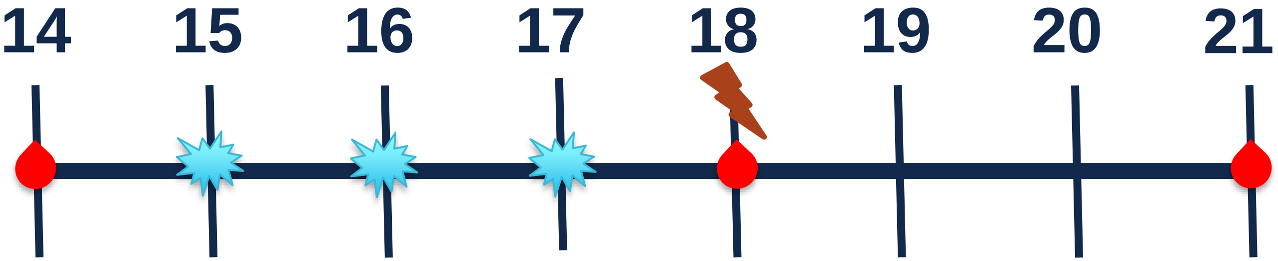
Dairy Focus Lab





Experimental Timeline



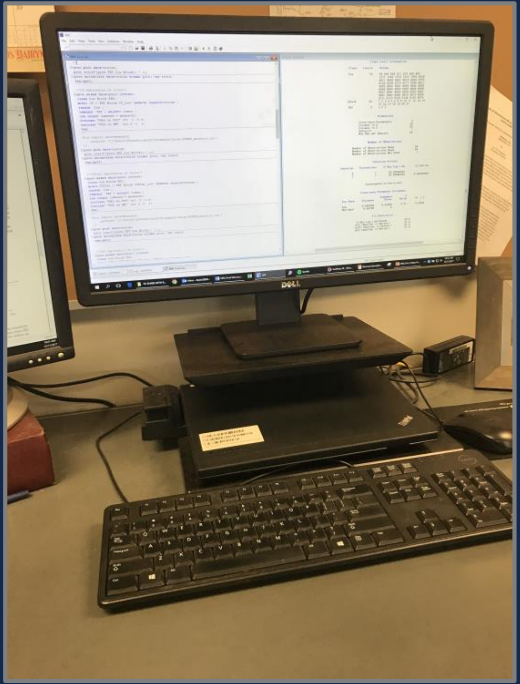
= **Blood** (chemical analysis)

= **Liver** (gene expression)

= **Aflatoxin challenge**

Daily
- Milk (3×)
- Dry Matter Intake
- Health checks

Statistical Analysis



SAS (v. 9.4, SAS Institute Inc., Cary, NC)

- MIXED and GLIMMIX Procedures

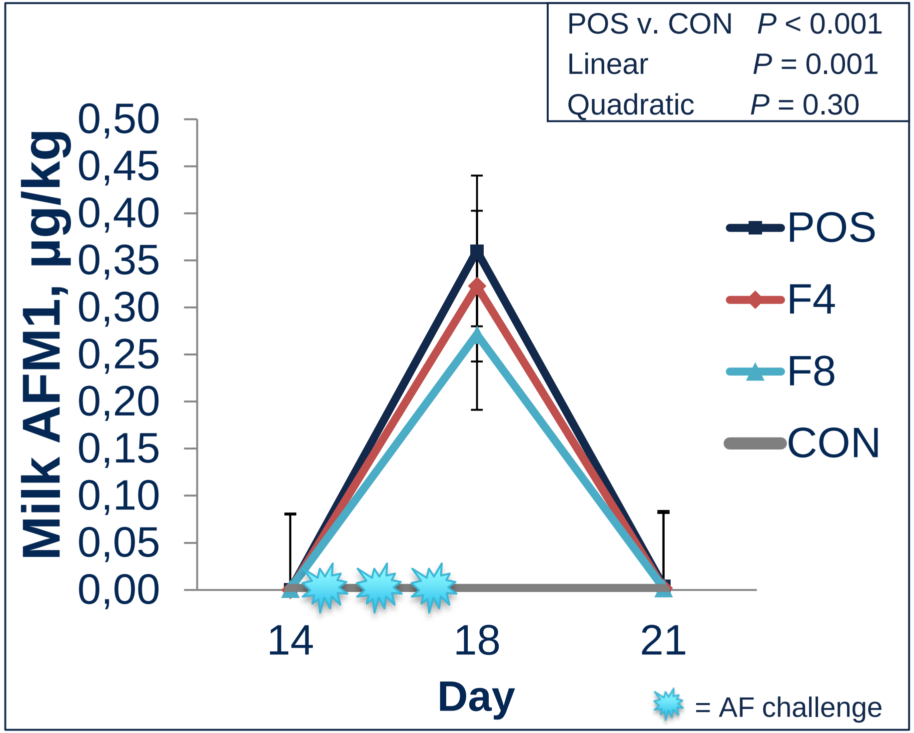
Orthogonal contrasts:

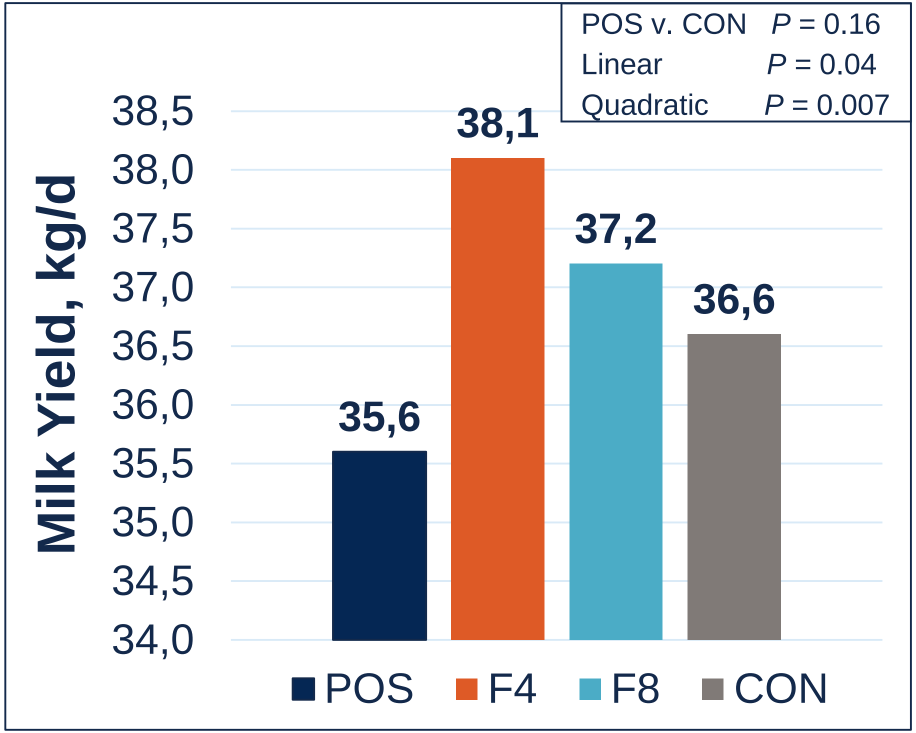
- POS vs. CON
- Linear and quadratic [POS (0), F4, and F8]

Significance declared at $P \leq 0.05$

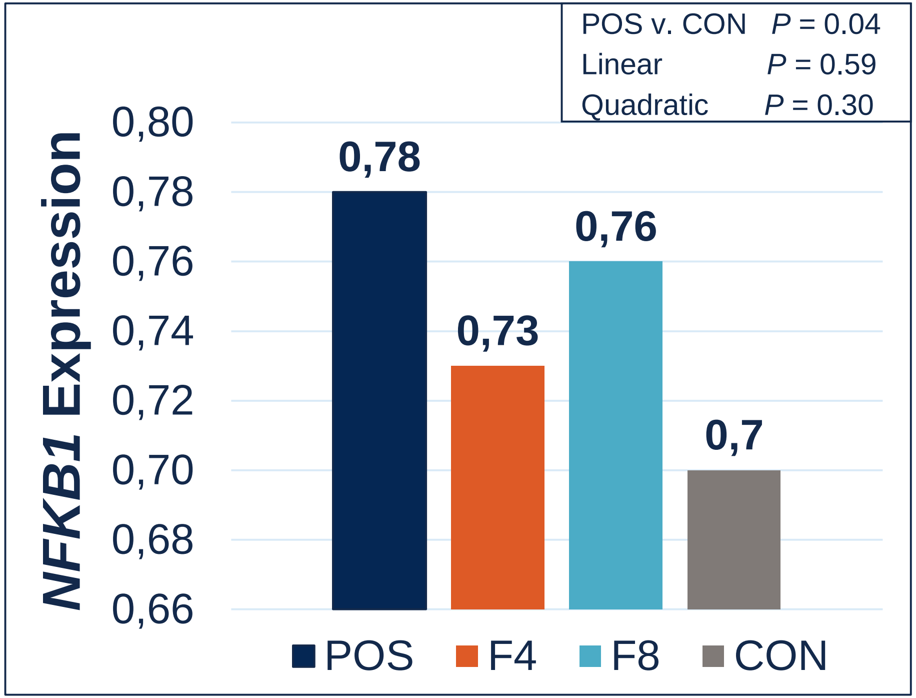
Trends declared at $0.05 < P \leq 0.10$

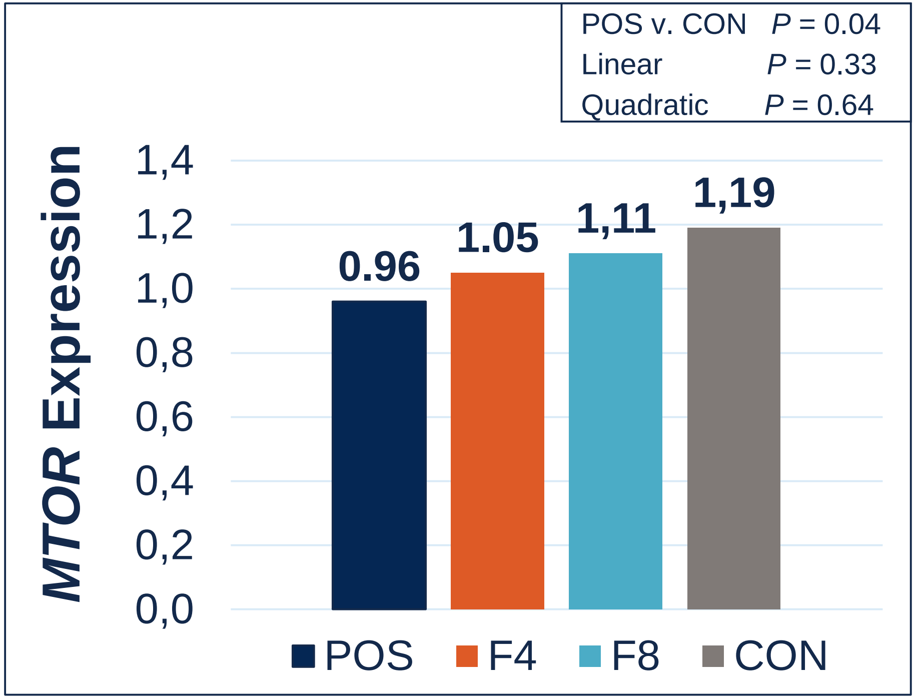
Aluminosilicate clay reduced milk AFM_1 and increased milk yield during aflatoxin challenge

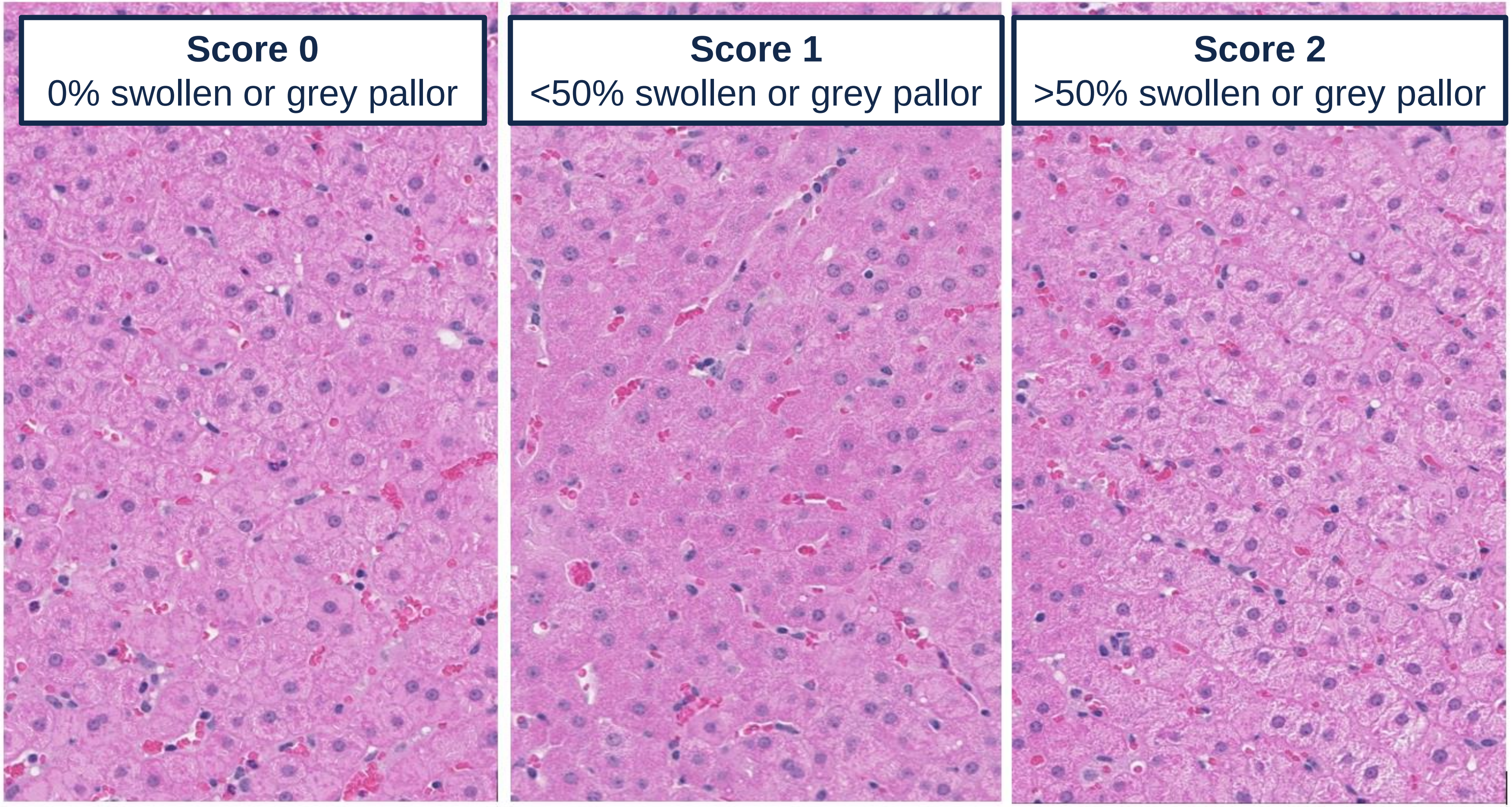


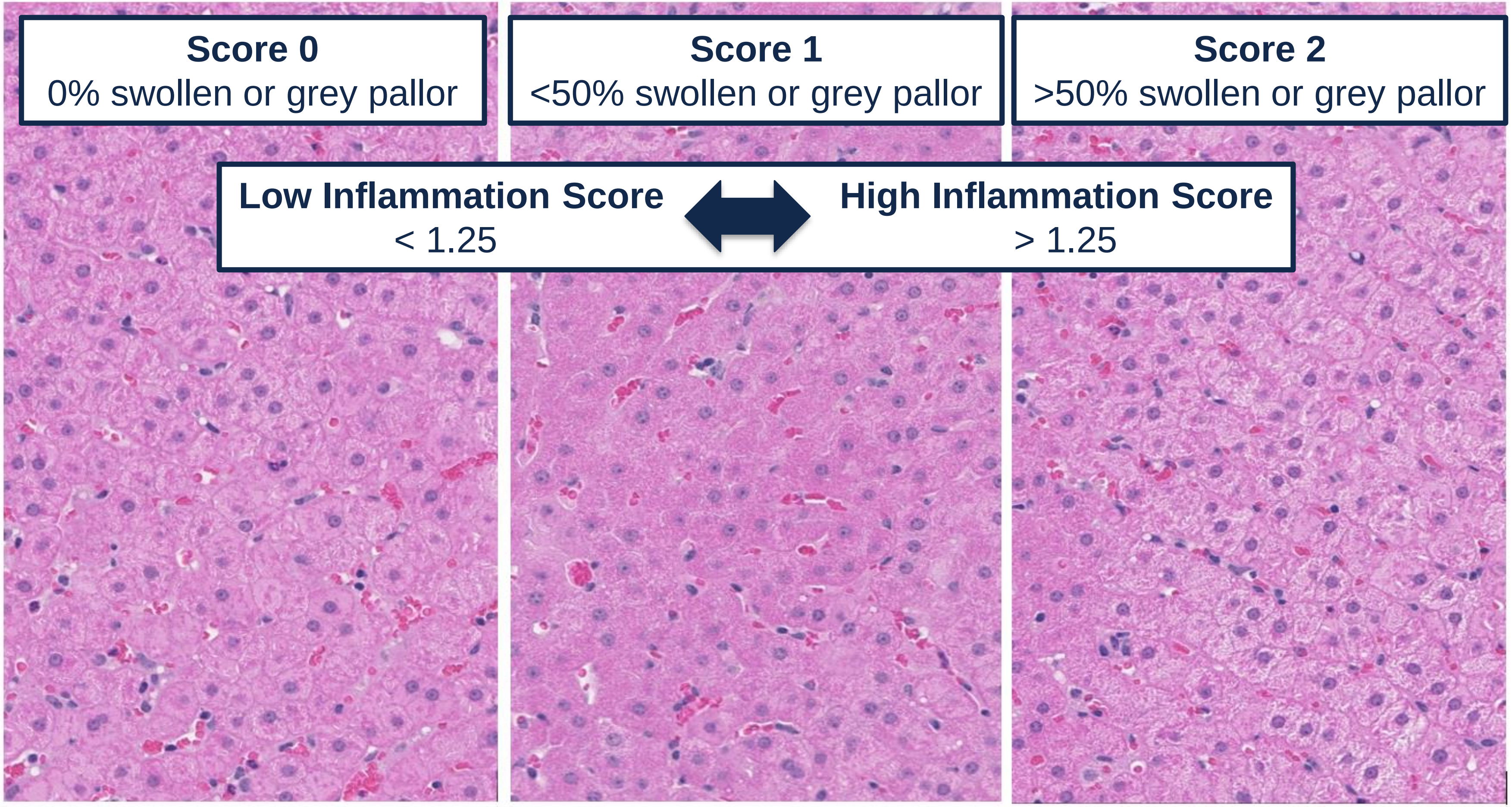


Aflatoxin challenge increased hepatic gene expression of *NFKB1* and decreased hepatic gene expression of *MTOR*



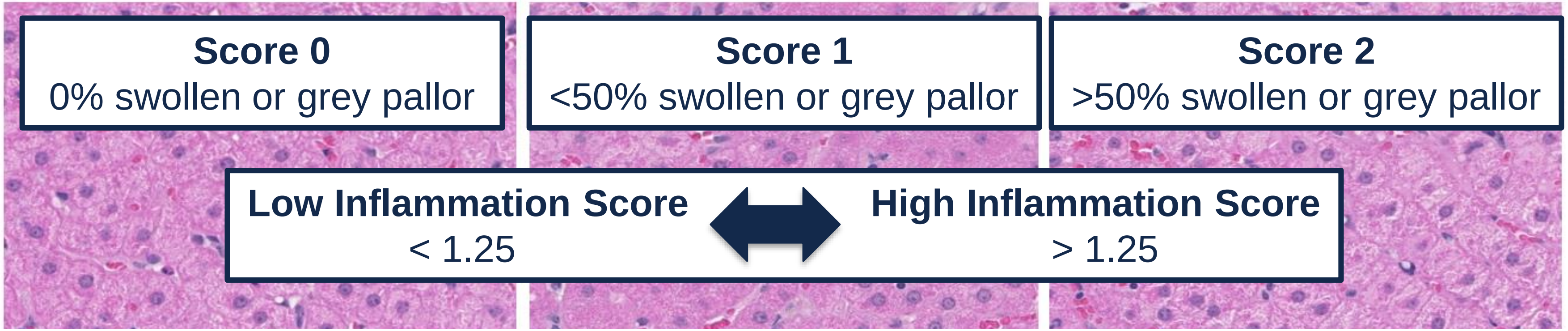



Score 0
0% swollen or grey pallor
Score 1
<50% swollen or grey pallor
Score 2
>50% swollen or grey pallor



Liver Histology

Ishak et al. (1995)



Score 0
0% swollen or grey pallor

Score 1
<50% swollen or grey pallor

Score 2
>50% swollen or grey pallor

Low Inflammation Score < 1.25 ⟷ **High Inflammation Score** > 1.25

Variable	Treatment	Level	OR	95 % CI	P-value
Hepatocyte inflammation	POS	POS-CON	5.14	0.97–27.33	0.05
	–	POS-F8	4.60	0.90–23.66	0.07
	–	POS-F4	2.60	0.51–13.27	0.25
	F4	F4-F8	1.77	0.39–8.11	0.46
	–	F4-CON	1.98	0.42–9.40	0.39
	F8	F8-CON	1.12	0.24–5.30	0.89

Liver Histology

Ishak et al. (1995)

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Outline

Introduction to aflatoxins

- Stress
- Mitigation strategies

Evaluation of mitigation strategies

- Injectable trace minerals
- Aluminosilicate clay

Conclusions

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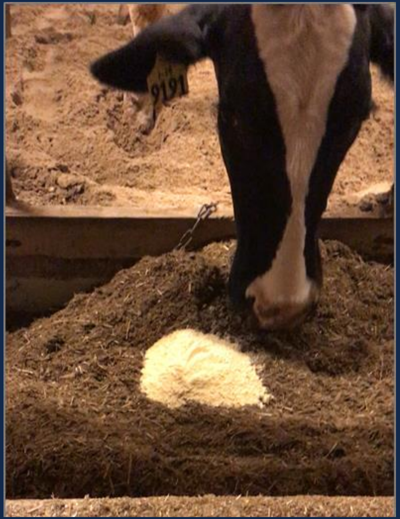


Conclusions

Aflatoxin negatively effects lactation performance

Injectable trace mineral neither hindered nor improved milk yield or milk aflatoxin concentration

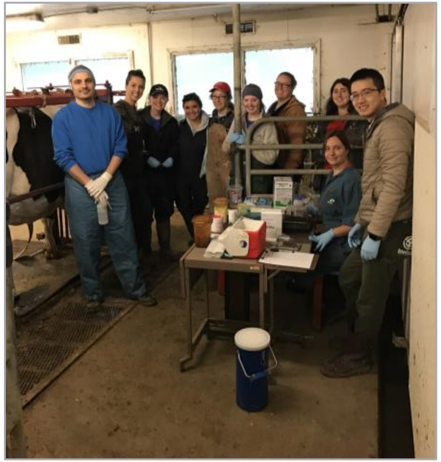
Aluminosilicate aided milk yield and milk protein yield and reduced aflatoxin excretion in milk

Aflatoxin caused liver inflammation and negatively affected blood markers and genes related to inflammation

Trace mineral injection aided in maintaining an adequate antioxidant response during AF challenge

Aluminosilicate clay aided in alleviating inflammation

Acknowledgements



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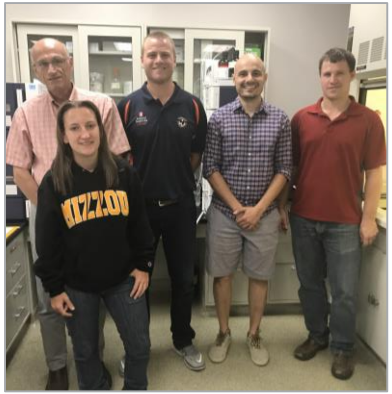

Dr. Lourens and Elfrieda Havenga

Dr. Devan Paulus Compart

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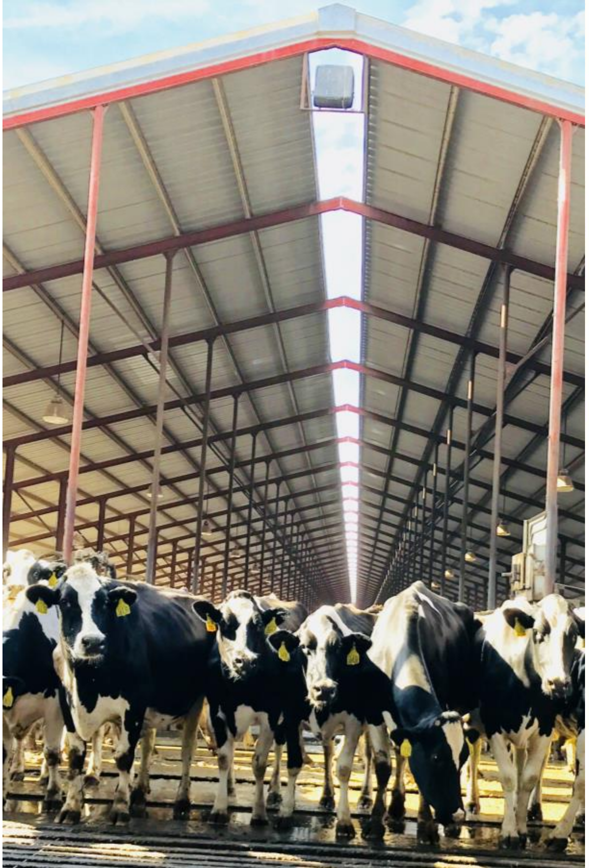

THANK YOU! QUESTIONS?

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