Can lactic acid bacteria bind aflatoxin B_1 in silage contaminated with the toxin?

Z. X. Ma[1], J. J. Romero[1], F. Amaro[2], K. C. Jeong[1], S. K. Williams[1] and A. T. Adesogan[1]

Department of Animal Sciences
University of Florida

Importance of Aflatoxin (AF)

- **AF from common feeds like corn, silages and wet feeds can be transferred to the milk of dairy cows**

- **AF causes up to 155,000 new cases of hepatocellular carcinoma every year (Wang and Tang, 2006).**

- **US economic losses due to mycotoxins are about $1.4 billion per year (CAST, 2003).**

- **The magnitude of decreases in performance due to subclinical aflatoxicoses are unknown**

Experiment 1
Can Silage Inoculant Bacteria Bind AFB_1?

Silage Inoculant Bacteria Selected

- *Lactobacillus plantarum R2014*
- *Lactobacillus plantarum EQ-12*
- *Lactobacillus plantarum PT5B*
- *Pediococcus acidilactici R2142*
- *Pediococcus acidilactici EQ-01*
- *Pediococcus pentosaceus EQ-44*
- *Pediococcus pentosaceus IA-38*
- *Lactobacillus buchneri R1102*
- *Propionibacterium jensenii SE-253*
- *Propionibacterium acidipropioni EQ-42*

Normal Role in Silage

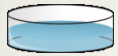
Rapid silage acidification and inhibition of DM losses

Inhibit yeast and mold growth and increase silage aerobic stability

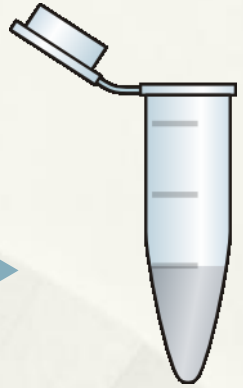
Material and Methods

Bacteria grown to 10^6 or 10^9 cfu/ml

Centrifugation



Bacteria pellet



5 µg/ml AFB_1 solution

AFB_1 concentration in supernatant analyzed by HPLC

Treatments were applied in quadruplicate;
AFB1 in bacteria and AFB1 blanks were quantified

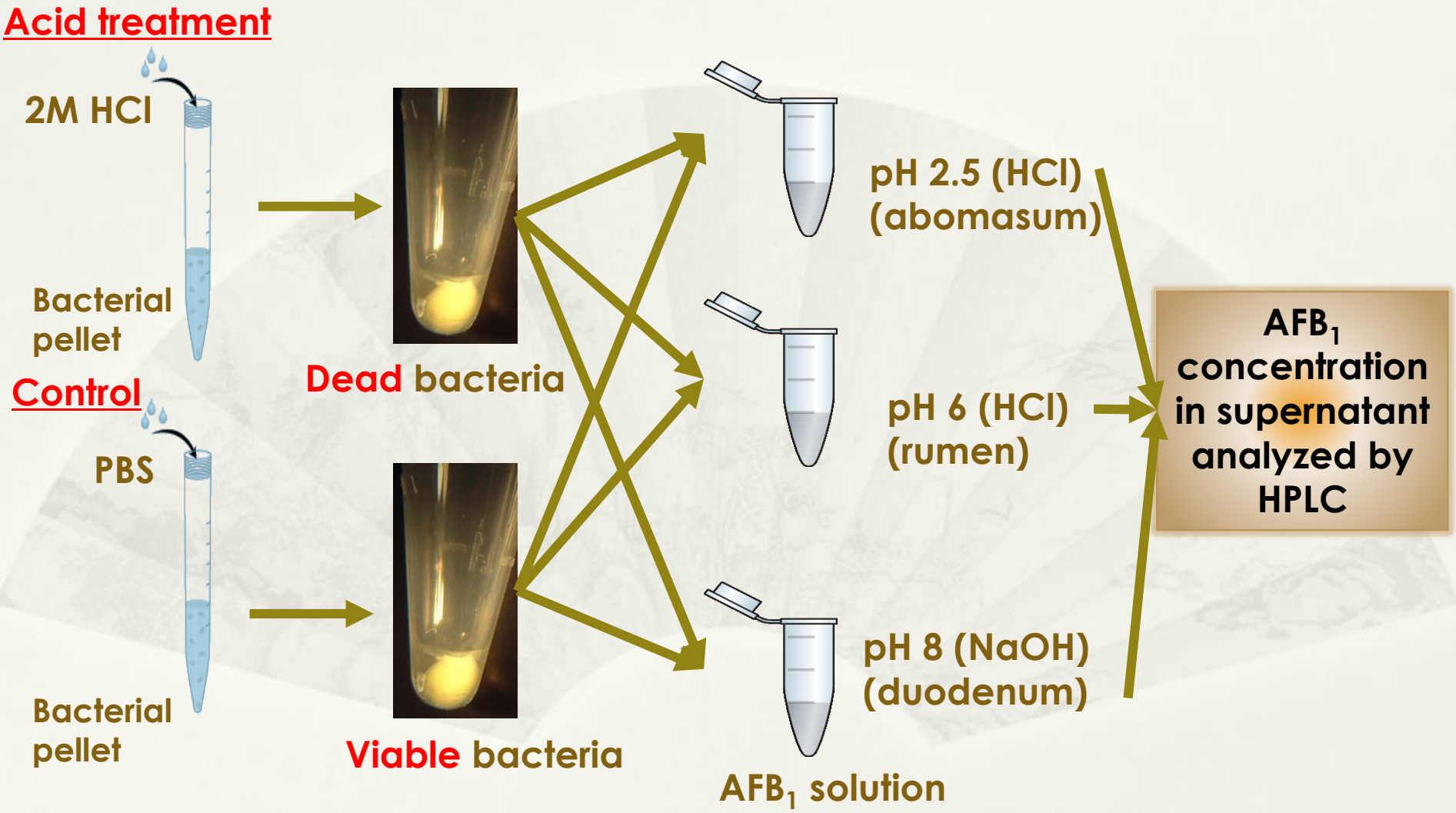
AFB1 binding by Two Populations of the Bacteria

Bacteria	Bacteria population, cfu/ml 10^6
	.....% AFB_1 bound.....
L. plantarum R2014	0.83[ab]
L. plantarum EQ12	1.08[ab]
L. plantarum PT5B	4.27[a]
L. buchneri R1102	0.04[ab]
Pe. acidilactici R2142	0.00[b]
Pe. acidilactici EQ01	0.66[ab]
Pe. pentosaceus EQ44	0.23[ab]
Pe. pentosaceus IA38	0.96[ab]
Pr. jensenii SE253	0.00[b]
Pr. acidipropionici EQ42	1.82[ab]
SEM	0.88
P value	0.04

[a, b, c, d] Means within a column with different superscripts differ, $P < 0.05$;
Initial AFB1 concentration = 5 µg/ml.

Experiment 2
Effects of Bacterial Species, Viability and pH on the Aflatoxin-binding Capacity of Silage Bacteria

Materials and Methods: Viability and pH Treatments



Treatments were applied in quadruplicate
AFB1 in Bacteria and AFB1 blanks were also quantified

Viability, Spp. and pH effects on AFB_1-binding

pH	L. plantarum		L. buchneri		P. acidilactici		SEM
	Viable	Dead	Viable	Dead	Viable	Dead	
	……AFB_1Bound, %……						
2.5	56.2^{b}	60.5^{a}	51.5^{b}	66.5^{a}	56.9^{a}	2.91^{bc}	0.57
6	10.1^{e}	15.8^{d}	1.47^{d}	34.1^{c}	6.76^{b}	4.40^{bc}	0.57
8	8.05^{e}	21.6^{c}	0.32^{d}	29.2^{c}	2.05^{c}	0.00^{c}	0.58
SEM	0.8	0.8	0.8	0.85	0.8	0.8	
Contrast *P* value[1]	L, Q	L, Q	L, Q	L, Q*	L, Q	Q*	

[1]Linear (L), quadratic (Q) effects ($P < 0.05$); Q* Quadratic trend ($P < 0.1$);
[a, b, c, d]Means within a bacterium with different superscripts differed, $P < 0.05$;
Effects of viability, spp. and the interaction differed, $P < 0.05$

Experiment 4
Can lactic acid bacteria bind aflatoxin B_1 in silage contaminated with the toxin?

Objective and Hypotheses

Objective: To determine effects of processing method (heat or acid treatment) on binding of AFB_1 by three silage bacteria.

Hypothesis 1: Inoculated silage bacteria would reduce the AFB_1 concentration of corn silage contaminated with the toxin

Hypothesis 2: Heat and acid treatment would increase the AFB_1-binding capacity of silage bacteria.

Treatments

1. Control

2. Toxin alone (30 µg of AFB1/ kg)

3. *L. plantarum (Lp)* (10^9 cfu/g) + toxin → Viable, Heated, Acid-treated

4. *L. buchneri (Lb)* (10^9 cfu/g) + toxin → Viable, Heated, Acid-treated

5. *P. acidilactici (Pa)* (10^9 cfu/g) + toxin → Viable, Heated, Acid-treated

Acid-treated (pH 2.5, 0.01 M HCl); Heated 85°C for 1h)

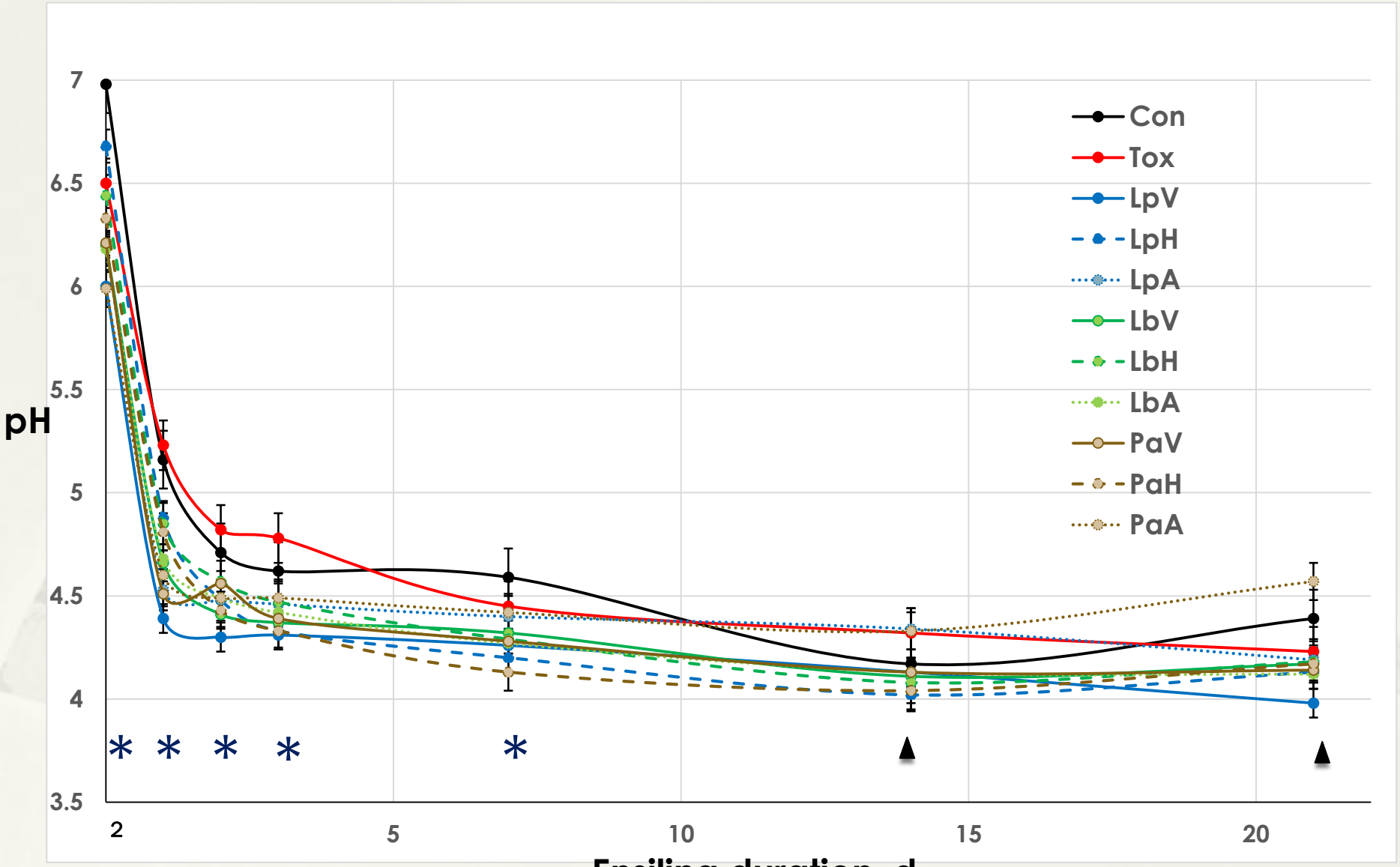
Materials and Methods

- Treatments were applied in quadruplicate to corn forage harvested at 35% DM and chopped to 1.9 cm
- Each replicate was ensiled (3 kg) in plastic bags
- AFB_1 concentration in silage fluid was measured at 0, 24, 48, and 72 h by HPLC on the silage fluid supernatant
- DM and pH measured at 0, 24, 48, 72 h, 7, 14, and 21 d
- Chemical composition measured at 0 h and 21 d

Chemical composition of corn forage before ensiling (% of DM or as stated)

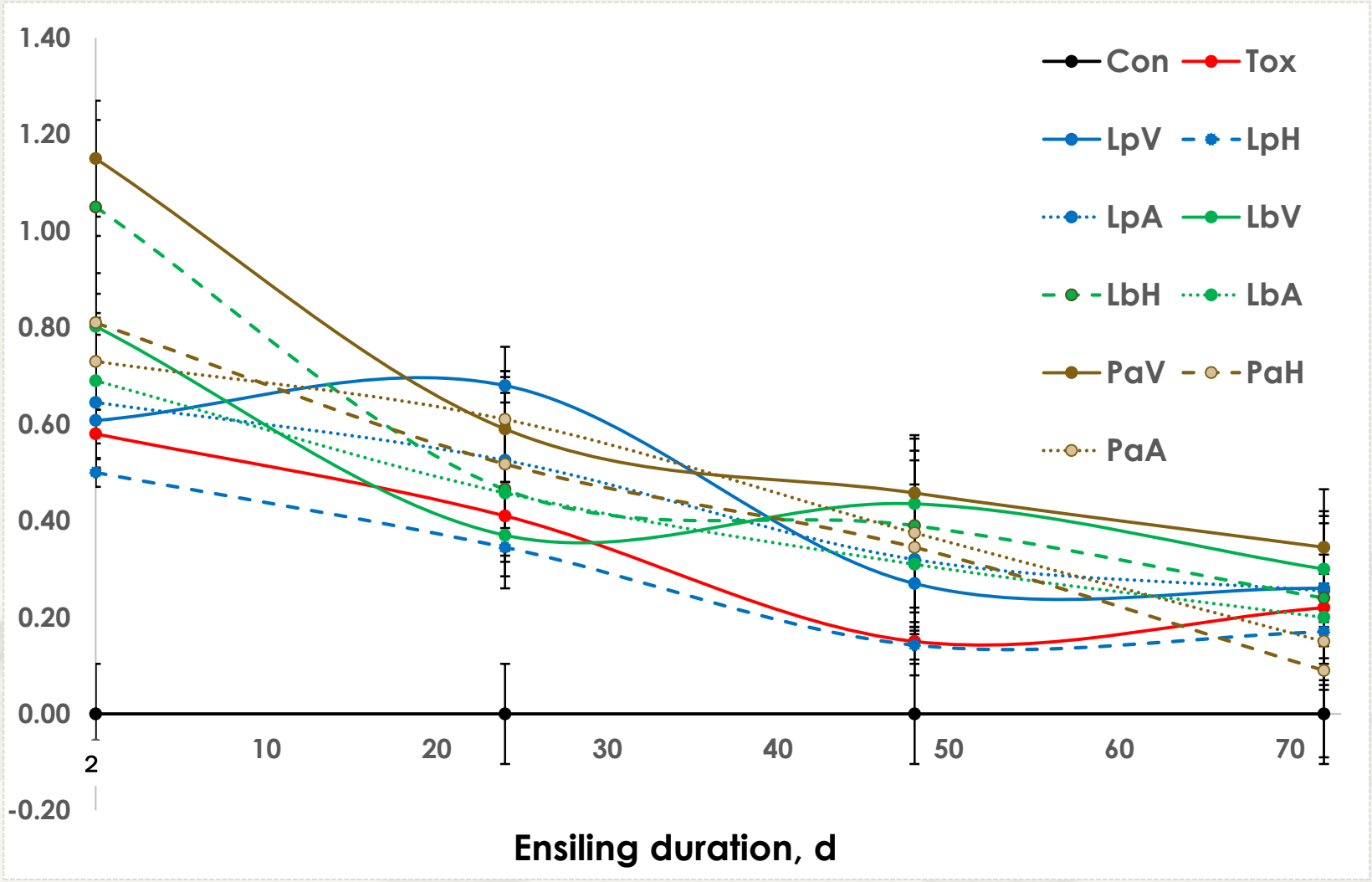
Treatment						
Additive	Processing method	DM, %	Ash	CP	ADF	NDF
Con		36.3	5.44	7.45	33.5	61.7
Toxin		36.4	4.46	7.18	32.1	60.3
Bacteria spp.						
	Viable	37.3	5.13	7.63	32.2	60.4
Lp	Heat	35.8	4.91	7.78	34.5	54.0
	Acid	37.1	4.82	7.93	29.6	55.2
	Viable	36.4	5.15	7.63	38.9	57.1
Lb	Heat	36.4	4.71	7.30	36.8	55.8
	Acid	33.6	4.87	7.18	28.8	61.3
	Viable	35.1	4.81	7.43	40.6	61.9
Pa	Heat	34.8	4.54	7.18	32.3	53.8
	Acid	34.0	4.81	7.53	32.1	64.8
SEM		1.01	0.22	0.2	2.71	2.83
P value		0.20	0.13	0.13	0.13	0.12

Change in pH during ensiling



▲ $0.05 < P < 0.1$ ∗ $P < 0.05$ Treatment x time, $P < 0.0001$ Error bars: SEM

Change in AFB_1 concentration during ensiling



- No treatment effects on the rates or intercepts ($P > 0.10$)
- AFB_1 concentration decreased linearly ($P<0.05$) in all treatments.

Treatment x time, *P* =0.22 Error bars: SEM

Chemical composition on d 21

Treatment				
Addi-tive	Proc[1]. method	DM	CP	NDF
Control (C)		36.0	8.83	55.2
Toxin (T)		32.7	8.25	58.9
Bacteria spp.				
Lp	Viable	34.6	8.48	50.4
	Heat	35.5	8.43	50.4
	Acid	35.5	8.30	46.7
Lb	Viable	34.6	8.20	50.7
	Heat	35.6	8.30	54.8
	Acid	33.9	8.43	50.9
Pa	Viable	34.8	8.28	53.5
	Heat	37.2	8.78	52.5
	Acid	34.7	8.03	50.2
SEM		0.78	0.18	1.92
P value		0.04	0.12	0.01

Contrasts	DM	CP	NDF
	P values		
C vs others	0.19	0.02	0.11
C vs T	0.01	0.03	0.18
T vs Lp	0.01	0.48	0.01
Lp proc.	0.36	0.62	0.44
T vs Lb	0.04	0.78	0.01
Lb proc.	0.91	0.47	0.38
T vs Pa	0.001	0.61	0.01
Pa proc.	0.26	0.58	0.36

[1]Proc., processing

Treatment had no effect on ash and ADF (P >0.1); data not shown

Fermentation parameters on d 21

Treatment . Additive	Proc. [1] Method	Lactic	Butyric
Control (C)		1.52	0.37
Toxin (T)		1.17	0.62
Bacteria spp.			
Lp	Viable	3.97	0.09
	Heat	2.77	0.09
	Acid	2.55	0.10
Lb	Viable	0.80	0.12
	Heat	2.38	0.14
	Acid	0.78	0.09
Pa	Viable	2.37	0.13
	Heat	2.09	0.08
	Acid	0.52	0.18
SEM		0.38	0.05
P value		0.001	0.001

Contrasts	Lactic	Butyric
C vs others	0.31	0.001
C vs T	0.56	0.001
T vs Lp	0.01	0.001
Lp proc.	0.01	0.90
T vs Lb	0.77	0.001
Lb proc.	0.10	0.89
T vs Pa	0.34	0.001
Pa proc.	0.03	0.94

[1]Proc., processing

Treatment had no effect on NH_3N, acetic and propionic acids (P >0.1); data not shown

Conclusions

- AFB1 can be bound by LAB but the effect depends on the bacterial population, species, strain and viability and the prevailing pH.

- The concentration of the AFB1 added to corn silage decreased linearly with time.

- Inoculated bacteria species or processing did not increase AFB1 binding in corn silage

Conclusions-Cont.

- Relative to the Control, AFB_1 decreased the CP and DM concentrations and increased the butyric acid concentration.

- Inoculation with bacteria prevented the increase in butyric acid and the decrease in DM.

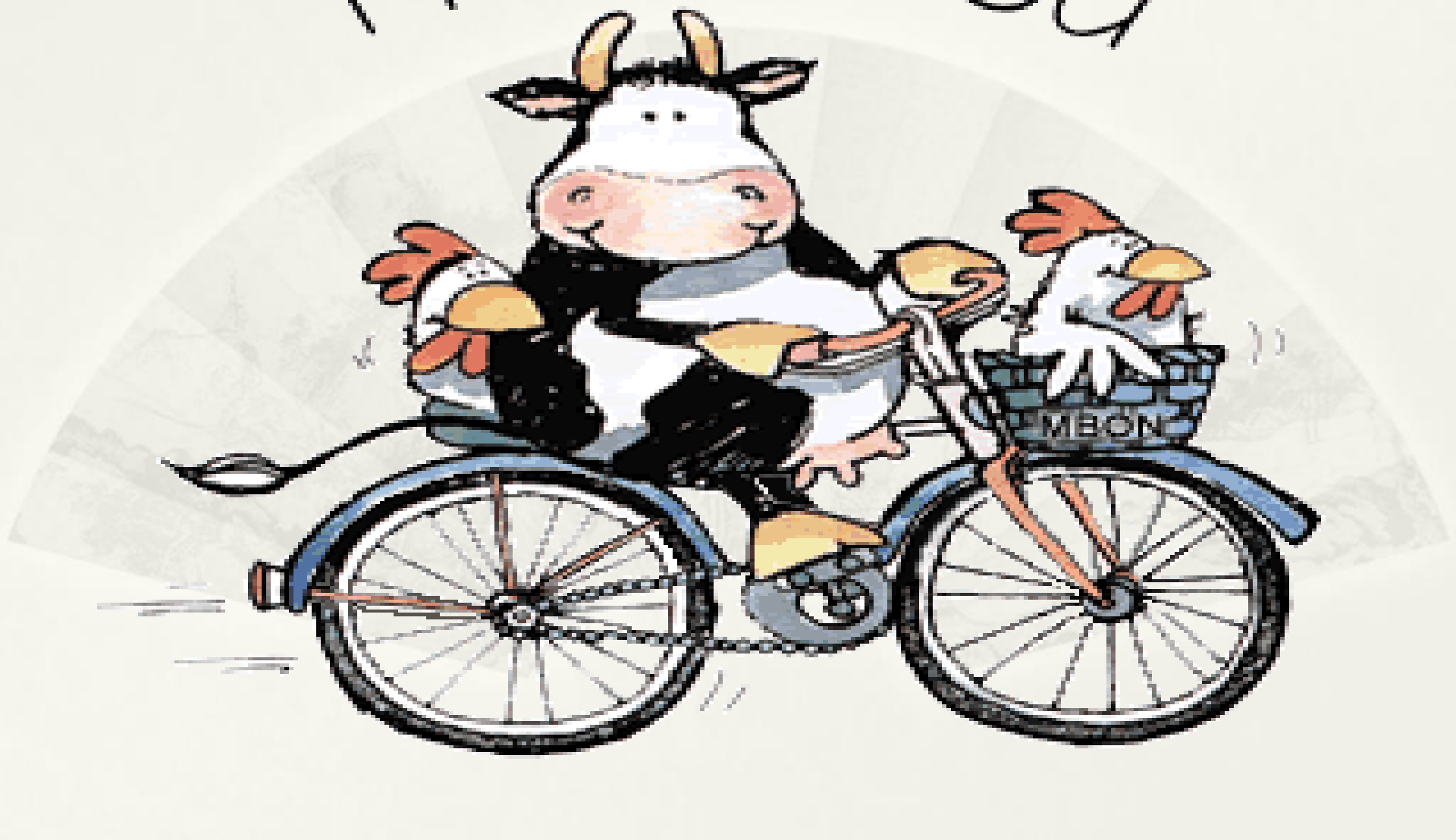
- Viable L. plantarum increased the rate of pH reduction and the lactic acid concentration relative to Control and toxin treatments

Acknowledgement

➢We gratefully acknowledge funding of this study by Lallemand Animal Nutrition

Thanks

Thank you



DM concentration (%) after different ensiling periods

Treatment		Ensiling duration, d								
Addi-tive	Process. method	0	1	2	3	7	14	21	SEM	P. contrast[1]
Con		36.3	34.7	34.2	37.2	38.1	38.7	36.0[ab]	1.26	Q
Toxin		36.4	34.8	34.2	35.6	34.8	34.7	32.7[b]	0.75	L
Bacteria spp.										
Lp	Viable									
	Heat									
	Acid									
Lb	Viable									
	Heat									
	Acid									
Pa	Viable									
	Heat									
	Acid									
SEM		1.01	0.87	1.21	0.91	1.08	1.00	0.78		
P value		0.2	0.2	0.06	0.32	0.05	0.09	0.04		

[a, b] Means within a column with different superscripts differ, $P < 0.05$;
[1]Linear (L), quadratic (Q) effect ($P<0.05$); L*, Linear trend ($P < 0.1$);

pH after different ensiling periods

Treatment		Ensiling duration, d								
Addi-tive	Process. method	0	1	2	3	7	14	21	SEM	P. contrast[1]
Con		6.98^{a}	5.16^{a}	4.71^{ab}	4.62^{ab}	4.59^{a}	4.19	4.39	0.14	L, Q
Toxin		6.50^{abc}	5.23^{a}	4.82^{a}	4.78^{a}	4.45^{ab}	4.21	4.23	0.12	L, Q
Bacteria spp.										
Lp	Viable	6.00^{c}	4.39^{c}	4.30^{c}	4.31^{c}	4.26^{ab}	4.14	3.98	0.07	L, Q
	Heat	6.68^{ab}	4.88^{ab}	4.48^{bc}	4.33^{bc}	4.20^{ab}	4.04	4.13	0.08	L, Q
	Acid	6.20^{bc}	4.53^{bc}	4.48^{bc}	4.46^{abc}	4.40^{ab}	4.25	4.19	0.10	L, Q
Lb	Viable	6.20^{bc}	4.66^{bc}	4.41^{bc}	4.37^{bc}	4.32^{ab}	4.13	4.17	0.06	L, Q
	Heat	6.44^{abc}	4.85^{ab}	4.57^{abc}	4.47^{abc}	4.29^{ab}	4.10	4.18	0.10	L, Q
	Acid	6.18^{bc}	4.68^{bc}	4.49^{abc}	4.42^{abc}	4.27^{ab}	4.15	4.12	0.07	L, Q
Pa	Viable	6.21^{bc}	4.51^{bc}	4.56^{abc}	4.39^{bc}	4.28^{ab}	4.15	4.14	0.06	L, Q
	Heat	6.33^{bc}	4.81^{ab}	4.43^{bc}	4.33^{bc}	4.13^{b}	4.06	4.17	0.09	L, Q
	Acid	5.99^{c}	4.60^{bc}	4.49^{abc}	4.49^{abc}	4.42^{ab}	4.35	4.57	0.09	L, Q
SEM		0.13	0.08	0.07	0.06	0.09	0.06	0.11		
P value		0.01	0.001	0.01	0.01	0.04	0.05	0.06		

[a, b] Means within a column with different superscripts differ, $P < 0.05$;
[1]Linear (L), quadratic (Q).

Chemical Composition at d 21

Treatment Additive	Process. method	DM, %	Ash	CP	ADF	NDF
Con		36.0	5.33	8.83	34.5	55.2
Toxin		32.7	5.12	8.25	34.9	58.9
Bacteria spp.						
Lp	Viable	34.6	4.80	8.48	33.0	50.4
	Heat	35.5	5.48	8.43	28.5	50.4
	Acid	35.5	4.62	8.30	29.3	46.7
Lb	Viable	34.6	5.08	8.20	33.5	50.7
	Heat	35.6	4.94	8.30	33.9	54.8
	Acid	33.9	4.69	8.43	34.1	50.9
Pa	Viable	34.8	5.26	8.28	33.7	53.5
	Heat	37.2	5.21	8.78	33.8	52.5
	Acid	34.7	4.75	8.03	36.0	50.2
SEM		0.78	0.20	0.18	2.76	1.92
P value		0.04	0.02	0.12	0.72	0.01

Contrasts	DM, %	Ash	CP	ADF	NDF
	P values				
Con vs others	0.19	0.08	0.02	0.63	0.11
Con vs Toxin	0.01	0.39	0.03	0.91	0.18
Toxin vs Lb	0.04	0.31	0.78	0.74	0.01
Toxin vs Lp	0.01	0.47	0.48	0.15	0.01
Toxin vs Pa	0.00	0.84	0.61	0.89	0.01
Lb viability	0.91	0.25	0.47	0.88	0.38
Lp viability	0.36	0.24	0.62	0.23	0.44
Pa viability	0.26	0.20	0.58	0.71	0.36

Fermentation Parameters at d 21

Treatment		pH	Ammonia, % of TN	Lactic, % DM	Acetic, % DM	Propionic, % DM	Butyric, % DM	Total VFA, % DM
Additive	Process. Method							
Con		4.39	2.18	1.52	2.97	0.52	0.37	6.52
Toxin		4.23	2.83	1.17	2.97	0.47	0.62	8.44
Bacteria spp.								
	Viable	3.98	2.65	3.97	3.50	0.33	0.09	11.7
Lp	Heat	4.13	2.50	2.77	3.34	0.36	0.09	10.3
	Acid	4.19	2.66	2.55	4.11	0.53	0.10	9.77
	Viable	4.17	2.27	0.80	2.90	0.48	0.12	7.08
Lb	Heat	4.18	2.09	2.38	2.38	0.42	0.14	7.81
	Acid	4.12	2.57	0.78	3.04	0.39	0.09	7.87
	Viable	4.14	3.20	2.37	3.34	0.35	0.13	8.51
Pa	Heat	4.17	2.11	2.09	2.28	0.32	0.08	6.92
	Acid	4.57	2.38	0.52	2.47	0.50	0.18	4.49
SEM		0.11	0.31	0.38	0.44	0.11	0.05	1.29
P value		0.06	0.33	0.001	0.15	0.87	0.001	0.02

Fermentation Parameters at d 21-cont.

Contrast	pH	Ammonia, % of TN	Lactic, % DM	Acetic, % DM	Propionic, % DM	Butyric, % DM	Total VFA, % DM
	P values						
Con vs others	0.08	0.29	0.31	0.89	0.39	0.001	0.20
Con vs Toxin	0.30	0.15	0.56	0.99	0.78	0.001	0.27
Toxin vs Lb	0.54	0.16	0.77	0.68	0.74	0.001	0.52
Toxin vs Lp	0.29	0.54	0.01	0.14	0.61	0.001	0.12
Toxin vs Pa	0.63	0.46	0.34	0.55	0.53	0.001	0.18
Lb viability	0.92	0.88	0.10	0.73	0.56	0.89	0.62
Lp viability	0.17	0.86	0.01	0.67	0.42	0.90	0.26
Pa viability	0.10	0.02	0.03	0.05	0.65	0.94	0.06