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Control of Regulated and Emerging Silage Mycotoxins

For info, #SafetyOfSilage on social media

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What are «Mycotoxins»?

Mycotoxins are defined as **molecules of low molecular weight** produced by fungi that elicit a toxic response through a natural route of exposure both in humans and animals.

They are often **very stable molecules** and **all are secondary metabolites** of molds belonging to several genera, in particular ***Aspergillus***, ***Alternaria***, ***Fusarium***, and ***Penicillium*** spp.

Other genera, such as *Chaetomium*, *Cladosporium*, *Claviceps*, *Diplodia*, *Myrothecium*, *Monascus*, *Phoma*, *Phomopsis*, *Pithomyces*, *Trichoderma* and *Stachybotrys*, include **mycotoxigenic species**.

To date, there are more than **22'000 fungal secondary metabolites** described in Antibase2021, but only a **restricted number has received scientific interest** from the 1960s and onwards

Scientific interest on Mycotoxins in forage from *Silage Review* (JDS, 101) of 2018

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J. Dairy Sci. 101:4020–4033
https://doi.org/10.3168/jds.2017-13909

Silage review: **Interpretation of chemical, microbial, and organoleptic components of silages[1]**

Limin Kung Jr.,*[2] R. D. Shaver,† R. J. Grant,‡ and R. J. Schmidt§
*Department of Animal and Food Sciences, University of Delaware, Newark 19711
†Department of Dairy Science, University of Wisconsin, Madison 53706
‡The William H. Miner Agricultural Research Institute, Chazy, NY 12921
§Lallemand Animal Nutrition, Milwaukee, WI 53218

"Measuring the pH and quantif... organic acids and alc... mentations. When d... mmonly quantifi... of nitrogenous compou...

Silage Evaluation

J. Dairy Sci. 101:3980–4000
https://doi.org/10.3168/jds.2017-13839

Silage review: **Recent advances and future uses of silage additives[1]**

R. E. Muck,*[2,3] E. M. G. Nadeau,† T. A. McAllister,‡ F. E. Contreras-Govea,§ M. C. Santos,# and L. Kung Jr.ǁ
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ǁDepartment of Animal and Food Sciences, University of Delaware, Newark 19716

"Unfortunately, in s... crop grain crops may c... and "Several possi... toxins include **micro**... compounds, **bindi**... at ensiling instead of at feeding, and other **enzymes** or **chemicals** to detoxify the mycotoxins."

Risk in silage and use of additives

J. Dairy Sci. 101:3952–3979
https://doi.org/10.3168/jds.2017-13837

Silage review: **Factors affecting dry matter and quality losses in silages[1]**

G. Borreani,*[2] E. Tabacco,* R. J. Schmidt,† B. J. Holmes,‡ and R. E. Muck§
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†Lallemand Animal Nutrition, Milwaukee, WI 53218
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§Retired, USDA, Agricultural Research Service, US Dairy Forage Research Center, Madison, WI 53706

"…the improper incorporation of deteri... n the top layers of the silo in the f... nation of the ration w... ntous fungi, aerobic... **ns**; and (2) could po... performance"

Silage management

Scientific interest on Mycotoxins in forage from *Silage Review* (JDS, 101) of 2018

J. Dairy Sci. 101:4034–4059
https://doi.org/10.3168/jds.2017-13788

Silage review: **Mycotoxins in silage: Occurrence, effects, prevention, and mitigation**[1]

I. M. Ogunade,*[2] C. Martinez-Tuppia,† O. C. M. Queiroz,‡ Y. Jiang,* P. Drouin,† F. Wu,§ D. Vyas,* and A. T. Adesogan*[3]
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Some **milestones** and **idea** for future research on Mycotoxin in Silage, reported by Ogunade et al. (2018):

- "Due to the ubiquitous occurrence of mycotoxins in grains and forages, **frequent contamination** of livestock feeds with **multiple mycotoxins**" can occur.
- "Exposure to dietary mycotoxins adversely affects the **performance and health of livestock**"
- "Forages can be contaminated with several mycotoxins in the field pre-harvest, during **storage, or after ensiling and during feed-out**."
- "Concerted research efforts should aim to identify or develop **silage additives** that are effective at increasing the fermentation, nutritional value, and aerobic stability of silage, but that also **sequester or degrade silage mycotoxins**"
- "future research also should develop **simple, rapid, and accurate mycotoxin detection techniques** to foster on-site or on-farm detection of mycotoxins in feeds"

Co-Occurrence of Regulated and Emerging Mycotoxins in Corn Silage: Relationships with Fermentation Quality and Bacterial Communities

Gallo et al. Toxins 2021, 13, 232.

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Material and Methods

Sixty-four dairy farms located in the Po Valley (Italy) and Sardinia were randomly selected and visited in the 2017–2019 harvest seasons to collect corn silage samples.

Corn silages were sampled at least **10-12 weeks after ensiling** from horizontal bunker silos

All corn silages were analyzed for the presence and concentrations of fungal metabolites by LC–MS/MS at the Department of Agrobiotechnology according to Sulyok et al. (2020). The analytical method has been extended to cover **more than fungal 500 metabolites**. Briefly, 5 g of sample was weighed and extracted with 20 mL acetonitrile/water/acetic acid (79:20:1, v/v/v) for 90 min on a rotary shaker (GFL, Burgwedel, Germany). Extracts were diluted in extraction solvent (ratio 1:1) and directly injected into the LC–MS/MS instrument.

To categorize the maize silage samples into their quantity and quality of mycotoxin contents, we used a **hierarchical cluster analysis** using main variables related to mycotoxin contamination (i.e., total count of mycotoxins and concentrations of **Aspergillus-, Fusarium-, Penicillium-, Alternaria-, and other mycotoxigenic fungi-produced mycotoxins**) by the unweighted pair group mean with the arithmetic averages (UPGMA) method by the CLUSTER procedure of SAS (2003).

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Table 1. Counts (*n*) and sums (µg/kg dry matter or DM) of mycotoxins of corn silages belonging to different clusters.

Items	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	√MSE	*p* Value
	n = 24	*n* = 22	*n* = 2	*n* = 9	*n* = 7		
Counts of mycotoxins	24.7	23.5	42.5	25.4	32.7	5.93	<0.05
Aspergillus toxins	3.1	2.6	4.0	2.2	4.1	0.99	<0.05
Alternaria toxins	1.0	0.2	2.5	0.3	1.1	1.07	<0.05
Zearalenoneand its metabolites	0.4	0.2	2.0	0.2	0.6	0.55	<0.05
Trichothecenes type B	0.8	0.7	1.5	1.0	0.9	0.56	0.256
Fumonisins and their metabolites	4.8	5.8	6.5	6.7	7.7	1.46	<0.05
Enniatins	0.8	0.3	3.5	0.2	1.0	1.18	<0.05
Beauvericin	0.8	1.0	1.0	1.0	1.0	0.24	0.133
Other Fusarium toxins	6.5	6.9	11.5	7.2	8.9	1.62	<0.05
Penicillium toxins	4.6	4.5	6.5	5.4	6.3	1.10	<0.05
Other fungi toxins	0.6	0.1	1.5	0.0	0.9	0.97	0.103
Unspecified fungi toxins	0.8	0.1	3.0	0.0	0.7	0.79	<0.05
Sums of mycotoxins							
Aspergillus toxins	147.0	84.5	565.2	70.3	186.7	104.04	<0.05
Alternaria toxins	5.8	4.4	18.7	29.6	18.7	32.67	0.308
Zearalenoneand its metabolites	8.8	4.0	152.8	0.5	41.4	46.27	<0.05
Trichothecenes type B	28.8	15.4	192.6	33.5	57.6	41.67	<0.05
FB and their metabolites	215.4	339.1	475.3	473.5	1944.9	289.56	<0.05
Enniatins	0.6	0.3	3.1	0.5	5.7	4.46	0.075
Beauvericin	4.1	8.5	30.8	19.7	27.1	13.15	<0.05
Other Fusarium toxins	229.9	755.3	619.7	1564.8	675.1	172.65	<0.05
Penicillium toxins	154.6	91.6	708.2	87.3	142.2	107.34	<0.05
Other fungi toxins	1.1	0.1	4.3	0.0	4.0	2.85	0.013
Unspecified fungi toxins	17.8	1.8	102.0	0.0	26.0	23.51	<0.05

√MSE: root mean square error. When not detectable, the limit of detection of specific mycotoxins was used to compute statistical analysis.

Table reproduced by Gallo et al. (2021)

Label of clusters:

cluster 1 (n = 24, defined as silages contaminated by low levels of both *Aspergillus-* and *Penicillium-produced* mycotoxins)

cluster 2 (n = 22, defined as silages contaminated by low levels of fumonisins and other *Fusarium*-produced mycotoxins)

cluster 3 (n = 2, defined as silages contaminated by high levels of *Aspergillus*-mycotoxins)

cluster 4 (n = 9, defined as silages contaminated by high levels of *Fusarium*-produced mycotoxins)

cluster 5 (n = 7, defined as silages contaminated by high levels of fumonisins and their metabolites)

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Gallo et al. Toxins 2021, 13, 232.

Regulated/Recommended Mycotoxins | Emerging Silage Mycotoxins

Aspergillus spp.

- **AFB1**
- **3-Nitropropionic acid, Kojic acid, Gliotoxin**,
- Averufin, Fumigaclavine C, Nigragillin, Siccanol, Versicolorin C

Alternaria spp.

- **Alternariol, Alternariol-methyl-ether, Tentoxin, Tenuazonic acid**
- Infectopyron, Macrosporin, Altersetin,

Fusarium spp.

- **DON, DON-3-glucoside, NIV, T-2 & HT-2, Fumonisin A1, A2, B1, B2, B3, B4, B6 and masked forms, phFB, hFB**, **ZEA, ZEA sulfate, Fusaric acid, Beauvericin & Enniatin A, A1, B, B1 and B2**
- Antibiotic Y, 7-Hydroxykaurenolide, Apicidin, Aurofusarin, Bikaverin, Butenolid, Culmorin, Epiequisetin, Equisetin, Moniliformin, Monocerin, Siccanol, Chrysogin, 15-Hydroxyculmorin,

Penicillium spp.

- **Mycophenolic acid, Roquefortine C, Marcfortine A**
- Flavoglaucin, Cyclopenin, Oxaline, Pestalotin, Phenopyrrozin, Questiomycin A, 7-Hydroxypestalotin, Secalonic acid, Andrastin A, Curvularin, Meleagrin, Quinolactacin A, Rugulosin

Other Fungal genera

- Ascofuranone, Ascochlorin, Barceloneic acid, Bassianolide, Calphostin, Chlorocitreorosein, Citreorosein, Fungerin, , Ilicicolin A, B, C, E, Rubellin D, Ternatin, Xanthotoxin

Ergot Alkaloids

- Ergocryptine, Ergocryptinine

Phytoestrogens

- Biochanin, Daidzein, Daidzin, Genistein, Genistin, Glycitein, Glycitin, Ononin, Coumestrol

Strategies for prevention and control of mycotoxins

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Many factors are involved in enhancing the formation of mycotoxins. They are **plant susceptibility** to fungi infestation, **suitability** of fungal substrate, **climate** conditions, **moisture** content and **physical damage** of seeds due to **insects and pests**.

Toxin-producing fungi may invade at **pre-harvesting period**, **harvest-time**, during **post-harvest handling** and in **storage**. According to the site where fungi infest grains, toxinogenic fungi can be divided into three groups: field fungi; storage fungi; and advanced deterioration fungi (Battilani et al., 2013; Nada et al., 2022; Ognuade et al., 2018).

Practices recommended to keep the conditions unfavorable for any fungal growth (FAO, 1997).

Primary level:

- development **of fungal resistant varieties** of growing plants;
- **control field infection** by fungi of planting crops;
- making schedule for **suitable** pre-harvest, harvest and post-harvest;
- **lowering moisture content** of plant seeds, after post harvesting and during storage;
- Store commodities at **low temperature** whenever possible;
- Using **fungicides** and preservatives against fungal growth;
- **Control insect infestation** in stored bulk grains with approved insecticides.

Secondary level:

- Stop growth of infested fungi by **re-drying** the products;
- **Removal** of contaminated seeds;
- **Inactivation** or **detoxification** of mycotoxins contaminated;
- **Protect stored products** from any conditions which favour continuing fungal growth.

Tertiary level:

- **Complete destruction** of the contaminated products;
- **Detoxification** or **destruction** of mycotoxins to the minimal level.

Recommendations to counteract risk of mycotoxins contamination in grains.
What about silage and other forage, in particular post-harvest?

Dynamic evolution of bacterial, yeast and fungal communities during ensiling of alfalfa silage and after exposure to air

Gallo et al. MycoKey - Bari, 9 to 12 November 2021

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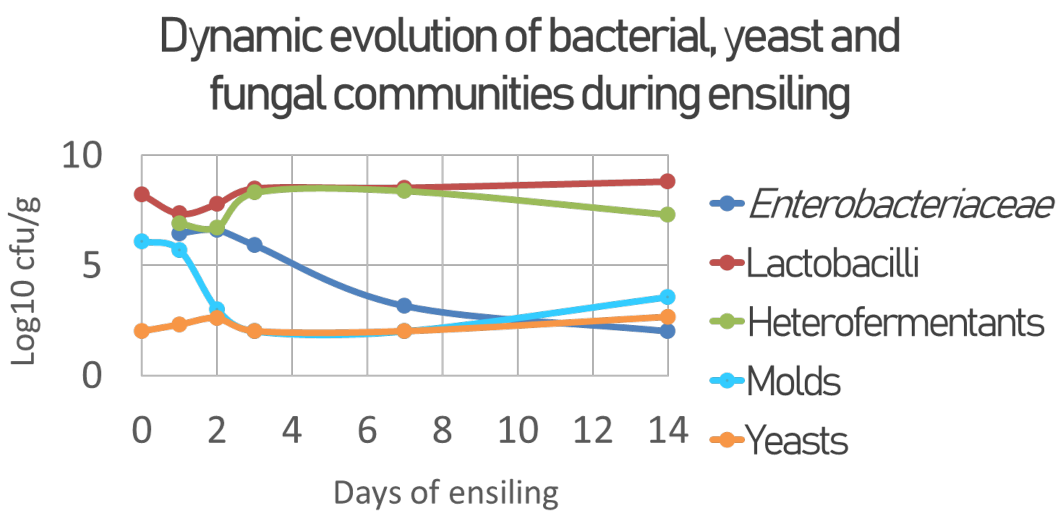
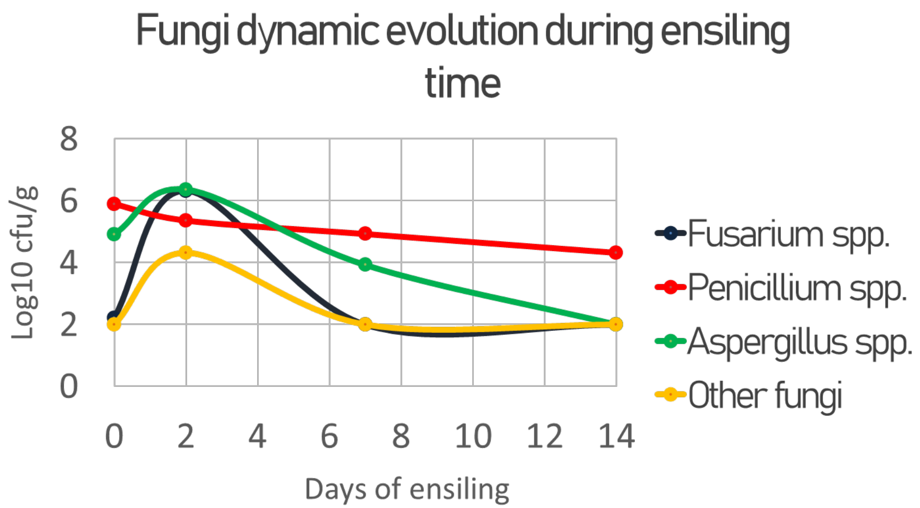
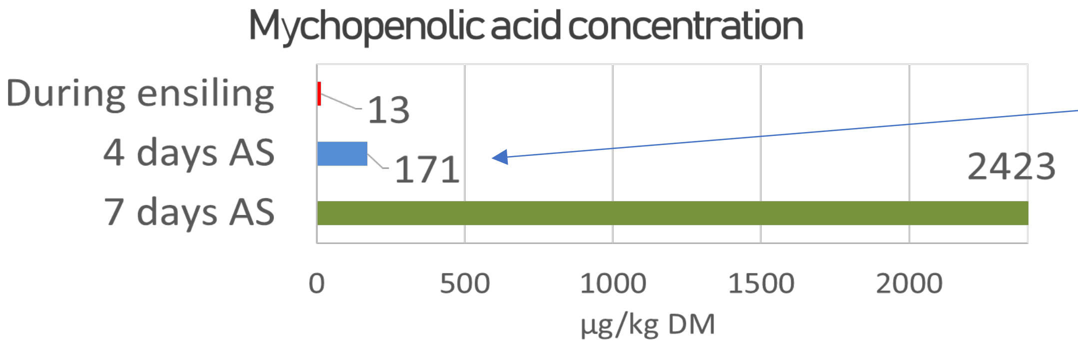




Mycotoxins:

- from an initial non-contaminated matrix, **DON was produced during ensiling phase**, up to 562 µg/kg DM
- **other Fusarium produced mycotoxins remained constants** (182 µg/kg DM for ZEA and 69 µg/kg DM for Fusaric Acid)



Silage exposed to air
Mycotoxigenic moulds are re-grown

Increase in aflatoxins due to *Aspergillus* section *Flavi* multiplication during the aerobic deterioration of corn silage treated with different bacteria inocula

Ferrero et al. 2018 JDS 102:1176–1193

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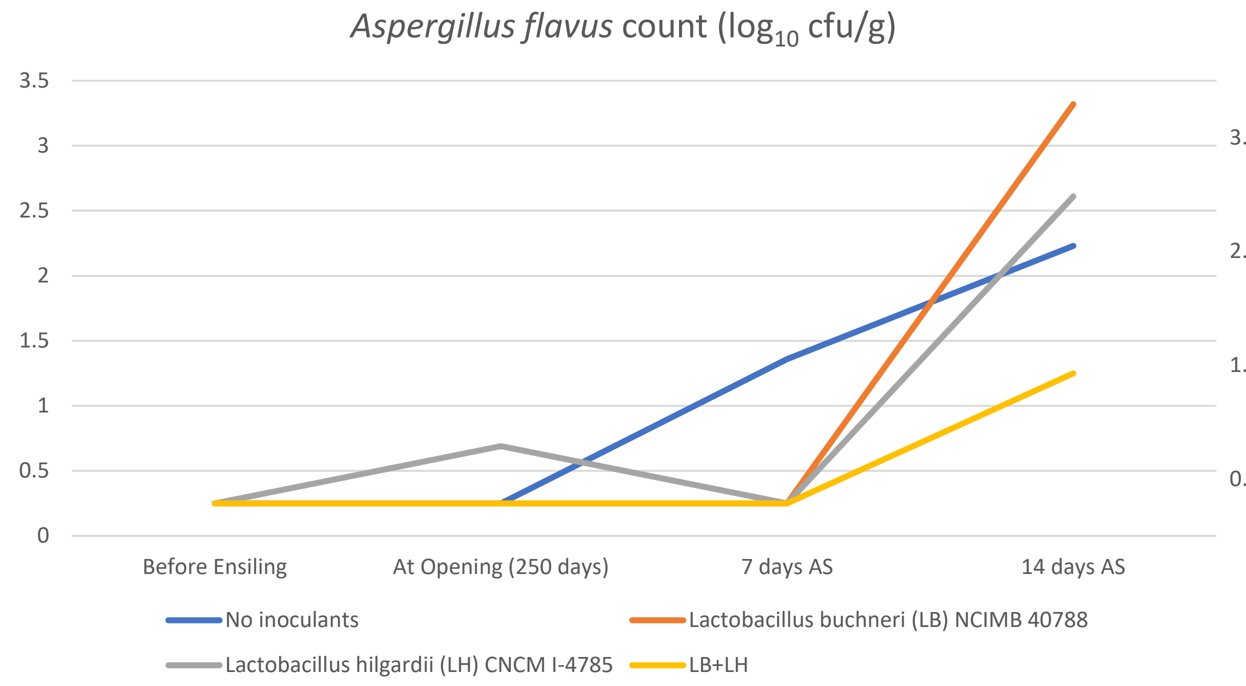
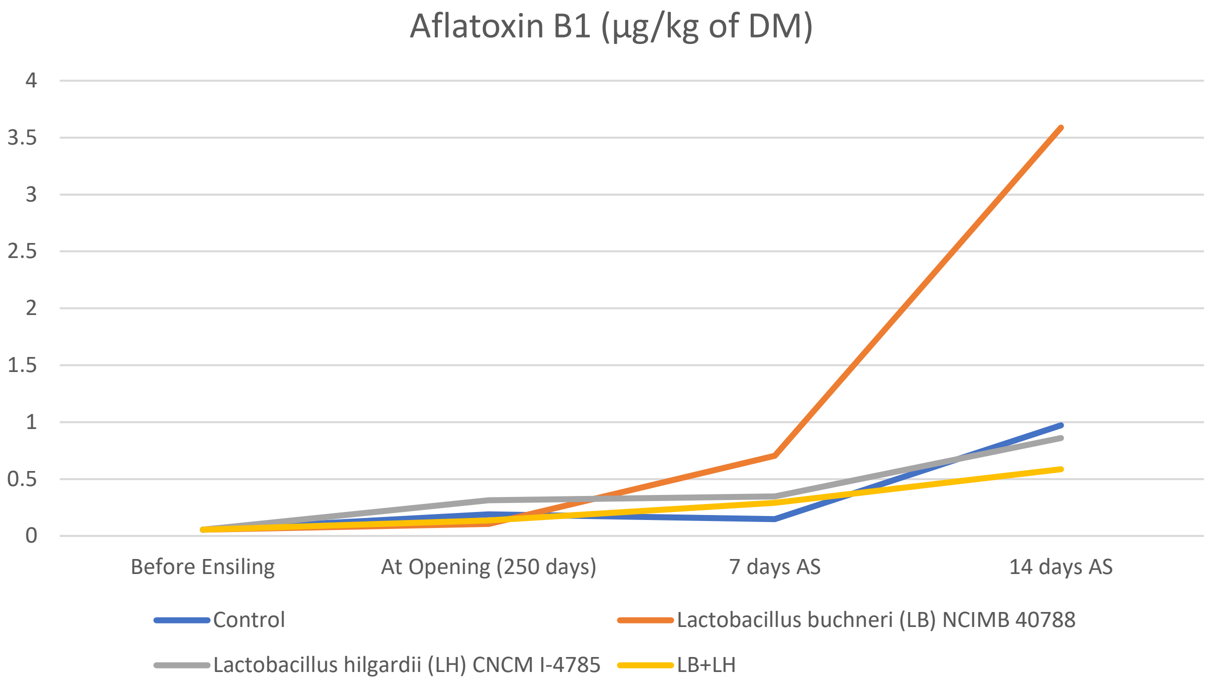
Aims of the work:

- **to evaluate whether the presence of *A. flavus* and aflatoxin production in corn silage originates from the field environment or growth of *A. flavus* takes place and additional aflatoxins are produced during storage or air exposure after silo opening** → Figures below were recalculated by tabular values.
- to evaluate the effect of different LAB inocula used to improve the aerobic stability of corn silage on reducing *A. flavus* growth and aflatoxin production during fermentation and air exposure → Results (Ctr 102d, LB 138d, LH 124d, LB+LH 365d)
- to characterize the toxigenic potential of *A. flavus* strains isolated from corn silages. → Results "Nine of 14 strains of *A. flavus* showed the presence of the complete gene pattern and, of these strains, 6 were able to produce aflatoxins".





Clostridium tyrobutyricum occurrence in silages and cattle feed: Use of molecular and simulation data to optimize predictive models

Mosconi M, Fontana A, Belloso Daza MV, Bassi D and Gallo A, 2023. Front. Microbiol. 14:1118646.

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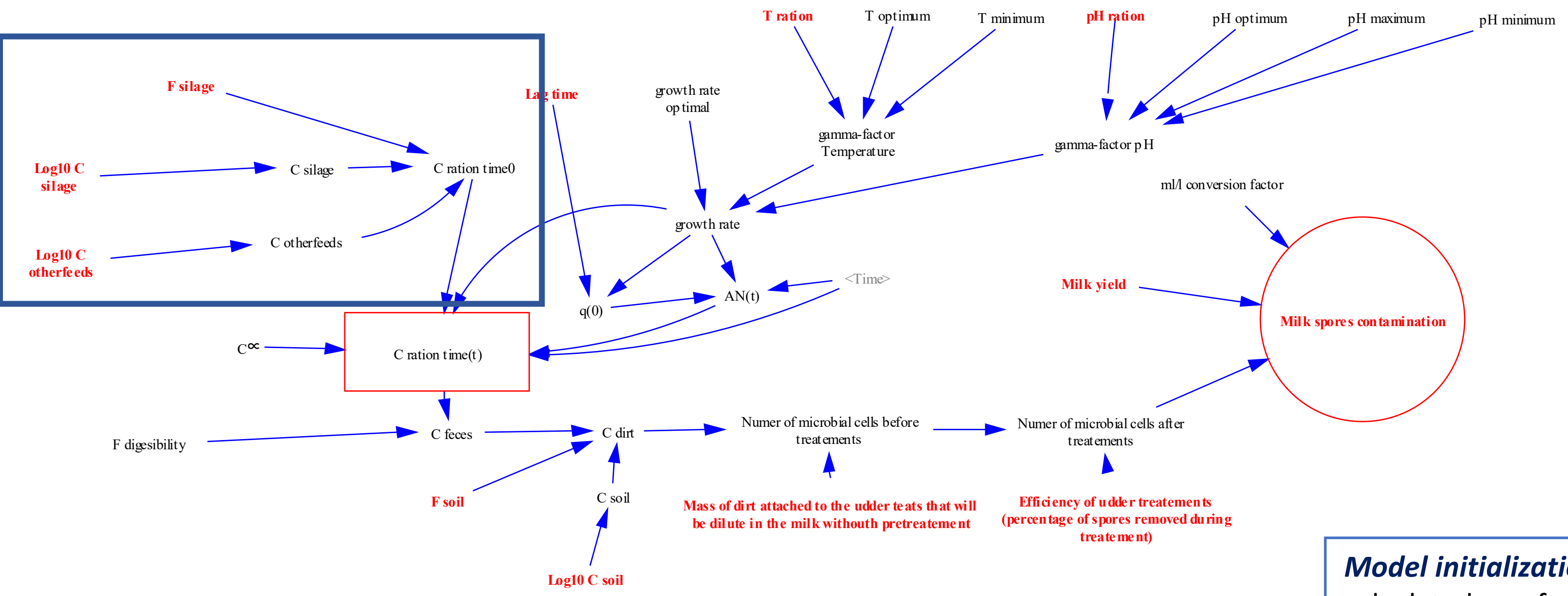
A study by Mosconi et al. (2023) examined the effect of **silage and total mixed ration (TMR) residues** on a reservoir of ***Clostridium tyrobutyricum*** contamination during successive TMR preparation



Model of Vissers et al. 2006 reported in Vensim® Professional, ver. 10.3.1

Unit operation	Equation
1. Mixing of silage and other feedstuffs resulting in the contaminated feed ration[1]	$C_{ration}(0) = F_{silage} \times C_{silage} + (1 - F_{silage}) \times C_{otherfeed}$
2. Storage of the feed ration resulting in growth during time t[2]	$\ln[C_{ration}(t)] = \ln[C_{ration}(0)] + \mu \times A_N(t)$ $-\ln\left(1 + \frac{e^{\mu \cdot A_n(t)} \times t}{e^{\ln(C_{\infty}/C_{ration}(0))}}\right)$ $A_n(t) = t + \frac{1}{\mu}\ln\left(\frac{e^{-\mu \cdot t} + q_0}{1 + q_0}\right)$ $q_0 = \frac{1}{e^{\lambda \cdot \mu} - 1}$ $\mu = \gamma(T) \times \gamma(pH) \times \mu_{opt}$ $\gamma(T) = \left(\frac{T_{ration} - T_{\min}}{T_{opt} - T_{\min}}\right)^2,$ $\gamma(pH) = \left[\frac{(pH_{ration} - pH_{\min}) \times (pH_{\max} - pH_{ration})}{(pH_{opt} - pH_{\min}) \times (pH_{\max} - pH_{opt})}\right]$

Reported by **Vissers et al. 2006**. J. Dairy Sci. 89:850–858

Model initialization: The contamination level of a carrier X after mixing ($C_{ration(0)}$, C_{dirt}) was calculated as a function of the contamination level of the preceding carriers (**C_{silage}, $C_{otherfeed}$**, C_{soil}, C_{feces}) and the fraction of these preceding carriers (F_{silage}, F_{soil}) in carrier X. All contamination levels (noted with C*) are in cfu/g and all fractions (noted with F*) are in %.

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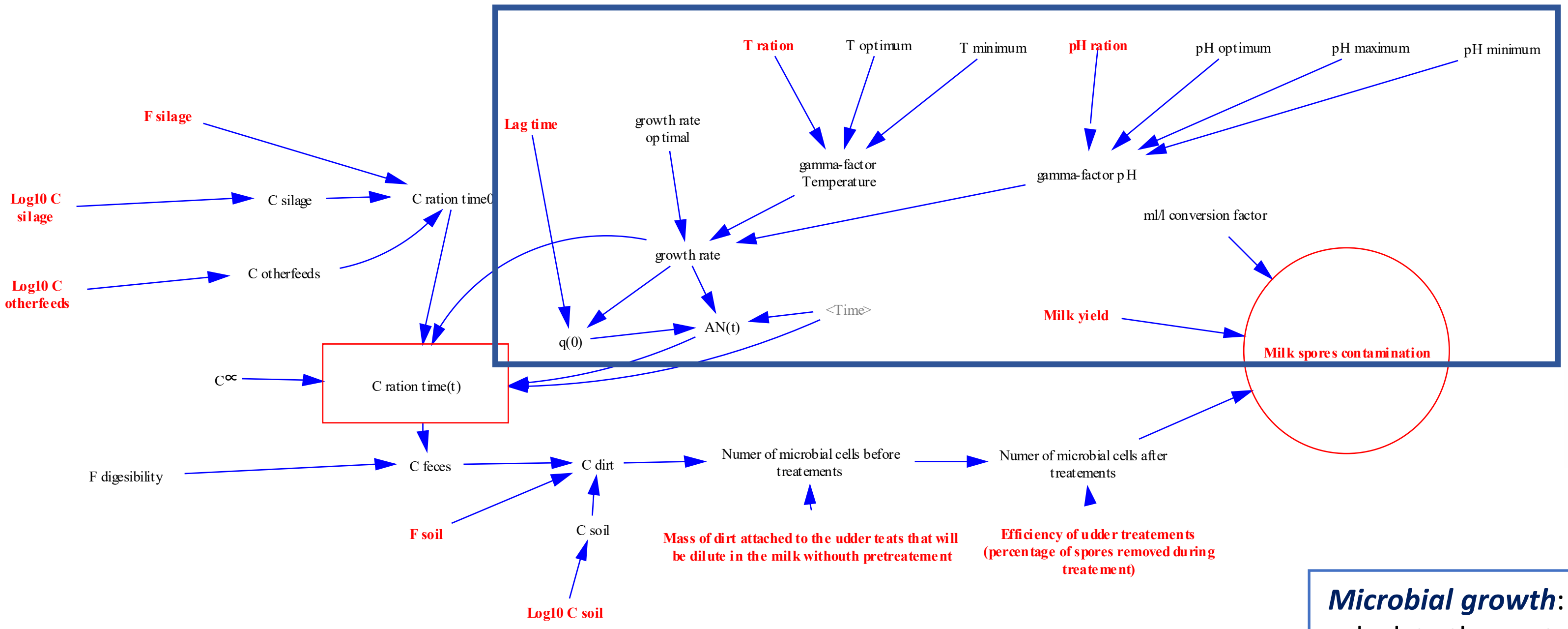

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Reported by **Vissers et al. 2006**. J. Dairy Sci. 89:850–858

Microbial growth: The Baranyi growth model (Baranyi and Roberts, 1994) was applied to calculate the contamination level of the feed ration after growth (**$C_{ration(t)}$**). Lag time λ (in h−1) equals 1/μ and t (h) is the time available for growth. The gamma-concept with γ-factors for temperature and pH was used to estimate the growth rate μ in h−1 (Zwietering et al., 1996).

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Vissers' model (2016) optimization and validation steps

E.g. Farm 1 – Summer 2019	Vissers (2016) parameters	Optimized parameters
Minimum growth temperature °C	9	9
Optimal growth temperature °C	37	37
Minimum pH required for growth	4,4	4,4
Optimal pH for growth	5,6	5,6
Maximum pH allowed for growth	6,8	6,8
Growth rate under optimal conditions h^{-1}	0,12	1,44
Time from sampling h	6	7
Lag time h^{-1}	0,1	0,1
TMR temperature °C	25	29
pH TMR	5,60	5,60
Predicted value CFU/g	2,83E+03	8,06E+06
Observed value CFU/g	8,06E+06	8,06E+06

E.g. Farm 2 – Winter 2021	Vissers (2016) parameters	Vissers (2016) parameters
Minimum growth temperature °C	9	9
Optimal growth temperature °C	37	37
Minimum pH required for growth	4,4	4,4
Optimal pH for growth	5,6	5,6
Maximum pH allowed for growth	6,8	6,8
Growth rate under optimal conditions h^{-1}	0,12	1,50
Time from sampling h	7	9,6
Lag time h^{-1}	0,1	0,1
TMR temperature °C	17,9	20
pH TMR	5,60	5,60
Predicted value CFU/g	2,39E+04	3,24E+05
Observed value CFU/g	3,24E+05	3,24E+05

The microbial growth rate under optimal condition parameter was modified including a wide range of values, from 0.12 h^{-1} (Vissers et al., 2006) to 0.43 h^{-1} (Ruusunen et al., 2012) to greater values.

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Expanded Vissers' model with silage and total mixed ration (TMR) residues

E.g. Farm 1 – Summer 2019	Vissers (2016) parameters	Optimized parameters	Expanded Vissers model (residues inclusion)
Minimum growth temperature °C	9	9	9
Optimal growth temperature °C	37	37	37
Minimum pH required for growth	4,4	4,4	4,4
Optimal pH for growth	5,6	5,6	5,6
Maximum pH allowed for growth	6,8	6,8	6,8
Growth rate under optimal conditions h^{-1}	**0,12**	**1,44**	**0,40**
Time from sampling h	**6**	**7**	**6**
Lag time h^{-1}	0,1	0,1	0,1
TMR temperature °C	**25**	**29**	**25**
pH TMR	**5,60**	**5,60**	**5,60**
Contamination of residues UFC/g	0,00E+00	0,00E+00	**4,17E+07**
Valore predetto UFC/g	***2,83E+03***	***8,06E+06***	***8,06E+06***
Valore reale osservato UFC/g	***8,06E+06***	***8,06E+06***	***8,06E+06***

The microbial growth rate under optimal condition parameter was modified including a wide range of values, from 0.12 h^{-1} (Vissers et al., 2006) to 0.43 h^{-1} (Ruusunen et al., 2012) to greater values.

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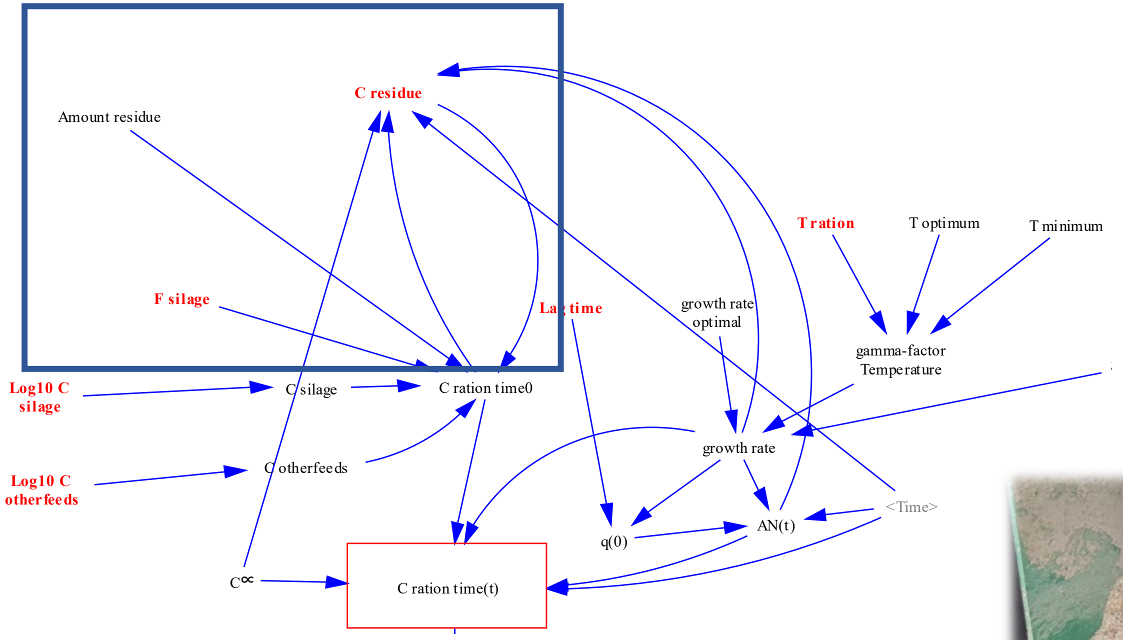
Vissers' model (2016) with silage and total mixed ration (TMR) residues



How to reduce the mycotoxins during ensiling? Are inoculants/additives efficient?

As reported by Ogunade et al. (2018):
"The degradation of mycotoxins into **nontoxic or less toxic metabolites** compared with the parent toxin can be achieved directly by enzymes or microorganisms that produce such enzymes (Loi et al., 2017)…
…but this depends by **a lot of environmental parameters**, including pH, temperature, availability of nutritive components, microorganism concentration, and availability of oxygen and other substrates (He and Zhou, 2010)"

Previously, Ma et al. 2017: "The objectives were to examine the aflatoxin B1 (AFB1)-binding capacity of silage bacteria and factors affecting the responses" - "This is the first study that used bacteria to reduce AF concentration in animal feed"

Experiment	Bacteria tested	Environmental conditions	AFB1-binding capacity
1	10	10^6 cfu/mL (in vitro medium)	Only L. plantarum (4%)
2	10	10^9 cfu/mL (in vitro medium)	All 10 bacteria, some from 24 to 33%.
3	3 viable and not viable (HCl-trated)	Different pH	From 0 to about 60%
5	3	Various viability-altering processes	Initial binding and linear reduction (safe level within 72h)

Discussion and Conclusion: - "The basis of AF binding by bacteria is **not well understood**" – "**Further studies** are required to examine binding of AFB1 in contaminated silage by silage bacteria and to elucidate the mechanisms involved in AFB1 binding to optimize the response."

Are bacterial inoculants efficient for reducing mycotoxins?

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Experiment	Bacterial Inoculants vs. CTR	Type of experiment	Effects on Mycotoxins	Notes
Gallo et al. 2018	• *L. buchneri* LB1819 & *Lactococcus lactis* O224	Mini-silos Corn silage	AFB1 ↓ (-32%) FB1 & FB2 ↓, MPA & Roq-C ↓ Fusaric acid ↑	Tested two silage densities (132 and 186 DM/m^3)
Saylor et al. 2020	• *E. faecium* • *L. plantarum* & *E. faecium* • *L. buchneri* LB1819 and *Lactococcus lactis* O224	Mini-silos Corn silage	AFB1 not detected Roq-C = Fusaric acid ↑ Altenuene ↓ Alternol monomethyl ether = Tentoxin =	Different length of storage (from 30 to 120 d)
Gallo et al. 2021	• *L. buchneri* LB1819 & *Lactococcus lactis* O224 • *E. faecium*, *L. plantarum*, *Lactococcus lactis* SR3.54, • *L. buchneri* and *L. plantarum* • *L. brevis* DSMZ 20054 • 2 *L. plantarum* • 3 *L. rhamnosus*	Mini-silos Corn silage	AFB1 ↓ (up to -53%) DON = or ↑ Tenuazonic acid ↓, MPA & Roq-C = or ↓ Fusaric acid ↑	Corn was inoculated with a toxigenic strain of *A. flavus* (ITEM 8069, 1 × 105 spores/mL) at silk emergence
Gallo et al. 2022	as above	Mini-silos High Moisture Corn	AFB1 = or ↑ DON = or ↓ Fusaric acid = or ↑	Corn was inoculated with a toxigenic strain of *A. flavus* (ITEM 8069, 1 × 105 spores/mL) at silk emergence

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Experiment	Bacterial Inoculants vs. CTR	Type of experiment	Effects on Mycotoxins	Notes
Ma et al. 2017	• *Lactiplantibacillus plantarum*; • *Lentiactobacillus buchneri*; • *Pediococcus acidilactici* (Viable, Heat, Acid)	Bags Corn silage	AFB1 ↓ (mainly!) or = or ↑	AFB1 added with a PBS solution Different ensiling duration (from 24 to 72h)
Ferrero et al. 2018	• *Lentilactobacillus buchneri* (LB) • *Lentilactobacillus hilgardii* (LH) • LB + LH	Mini-silos Corn silage (at opening - 250 days)	AFB1, AFB2 ↑ AFG1 ↓ or ↑ AFG2 ↓	The AF were found in all (predominance of AFB2)
Møller et al. 2021	• 4 *L. plantarum* • 2 *L. brevis* • 4 *Levilactobacillus* spp.	In vitro study	AFB1 ↓ (6) or ↑ (4) AFB2, AFG1, AFG2 ↓ (9) or ↑ (1) (after 24h of LAB inoculation) OTA and ZEN ↓ (up to 50%)	Tested Inhibition of *A. parasiticus* growth and mycotoxins production
Wang et al. 2022	• *L. buchneri* (LB) • L. plantarum (LP) • LB + LP	Large plastic silos (6 days of ensiling)	AFB1 and AFB2 ↓ ZEA ↓ DON = FB1 and FB2 = or ↑	Corn infected 2 times with *A. flavus* and *F. graminearum* spores
Guan et al 2023	• *L. plantarum* Q1-2 • *L. salivarius* Q27-2	Bags Alfalfa + concentrates (Mixed fermented feed, up to 30 days)	AFB1 ↓ (up to 34%) DON ↓ (up to 90%)	
Dong et al 2023	• *E. faecium* (E) • *E. faecalis* (M)	Minisilos Corn silage (up to 90 days)	DON & ZEA ↓ AFB1 ↓ (mainly M)	
Mateo et al. 2023	• *Leuconostoc mesenteroides* ssp. *Mesenteroides* (more efficient)	In vitro study (*Fusarium* spp. isolated from cereals.)	DON, ZEA, T2, HT2 ↓	The efficacy was higher at 20°C followed by 30 and 25°C

Are chemical additives efficient for reducing mycotoxins?

Experiment	Chemical additives vs. CTR	Type of experiment	Effects on Mycotoxins	Notes
Franco et al., 2022	• Formic acid-based additive (formic acid, propionic acid, sodium formate, and potassium sorbate); • Salt based additive (sodium nitrite, sodium benzoate, and potassium sorbate)	Mini-silos Grass silage (timothy and meadow fescue) 93 days of storage	DON ↓ ZEN ↓	3 ensiling management: • Loose compaction • Tight compaction • Tight compaction with soil + feces contamination
Kalúzová et al., 2022	• Urea	Bags Corn silage 165 days of storage	Aflatoxins n.d. DON ↑ Fumonisin B1 ↑ Nivalenol ↑ Ochratoxins n.d. ZEN ↓	The mycotoxin content after the addition of silage additives was not statistically significant, hence, their effect in corn silage was not confirmed
Alba-Mejìa et al., 2024	• Mixture of formic acid, propionic acid, benzoic acid, ammonium formate, sulfite ammonia caramel	Mini-silos Grass silage 90 days of storage	ZEN ↓ DON ↔	6 orchard grass varieties 2012 and 2013
Silva et al., 2024	• Sodium formate • Sodium chloride	Large plastic boxes 50-L Wet brewers’ grains 30 days of storage	Below the limit of quantification	

A Preliminary Study to Classify Corn Silage for High or Low Mycotoxin Contamination by Using Near Infrared Spectroscopy

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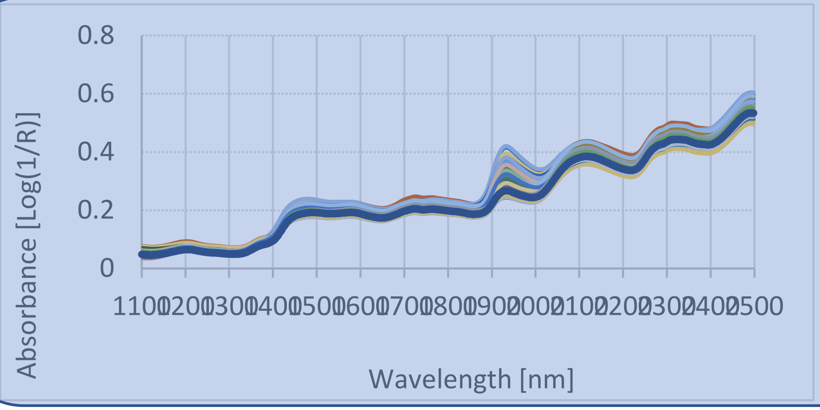
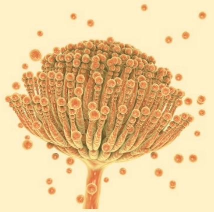
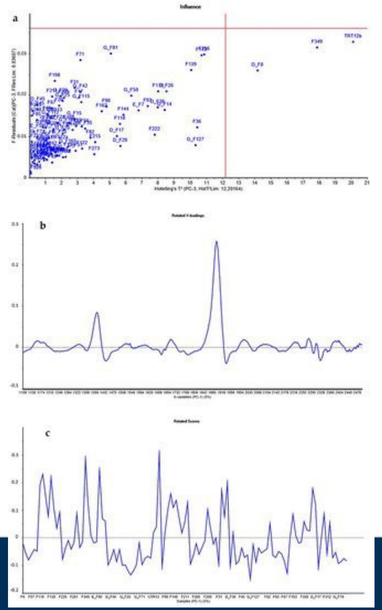


Corn silage (n=120) NIR spectra acquisition

Mycotoxin contamination

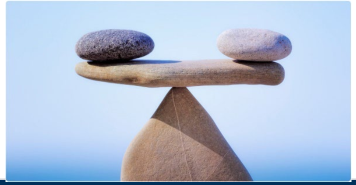


PCA: spectra outliers identification



Identification of cut-offs to QUALITATIVELY define sample classes based on the **level of contamination by concentration** (i.e., µg/kg dry matter) or **count** (i.e., n) of :

- ***All total detectable mycotoxins***;
- ***regulated and emerging Fusarium toxins***;
- **emerging *Fusarium* toxins;**
- ***Fumonisins and their metabolites;***
- ***Penicillium* toxins.**



Synthetic Minority Over-sampling Technique (SMOTE) algorithm in order to re-balance classes

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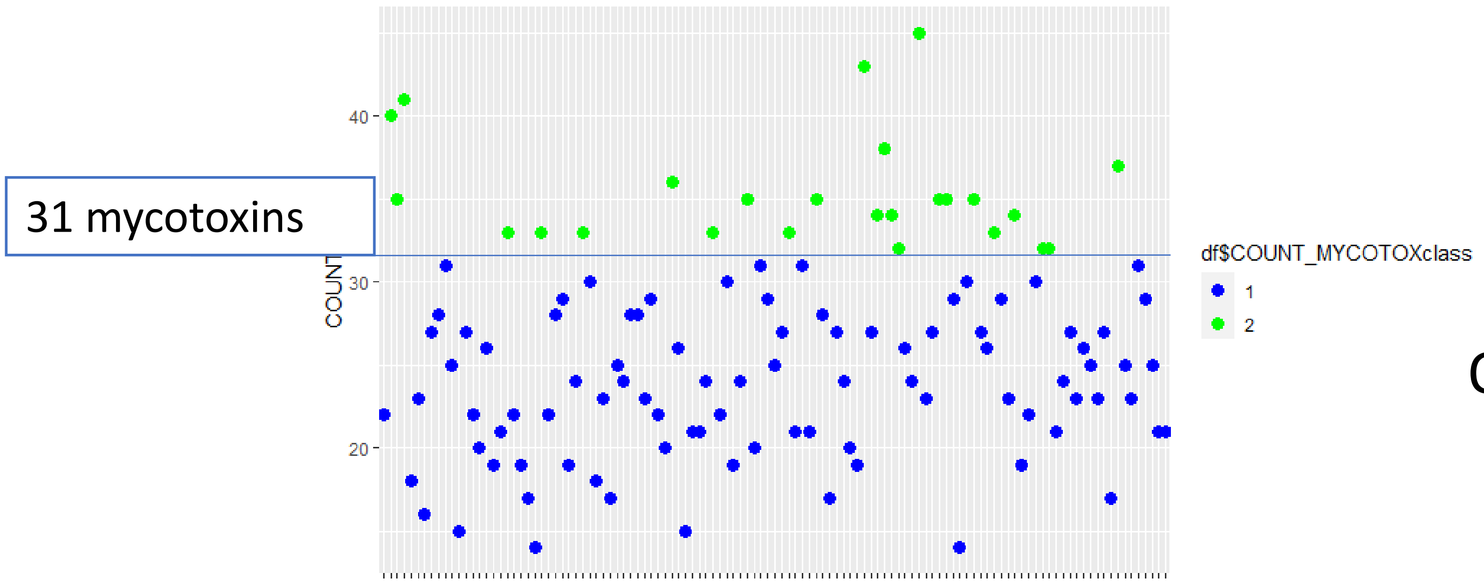
Random Forest NIR Calbration to grouped corn silage (n = 120) based on their mycotoxin contents and counts.



		Predicted		
		N1	N2	
Observed	N1	97%	3%	29
	N2	0%	100%	29
		28	30	

Take home message

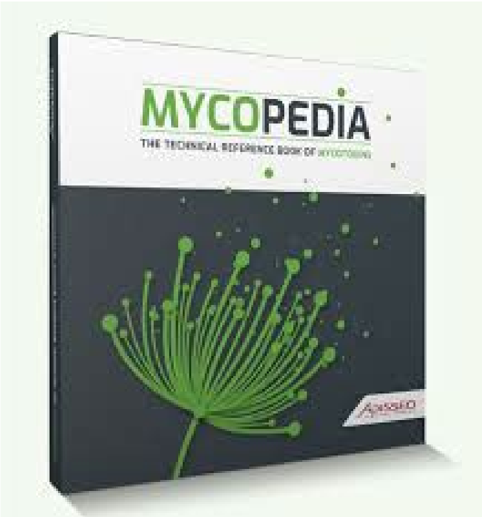
- There is a ubiquitous occurrence of mycotoxins in grains and forages. Silage can be **concomitantly** contaminated by a lot of **regulated and emerging mycotoxin**.

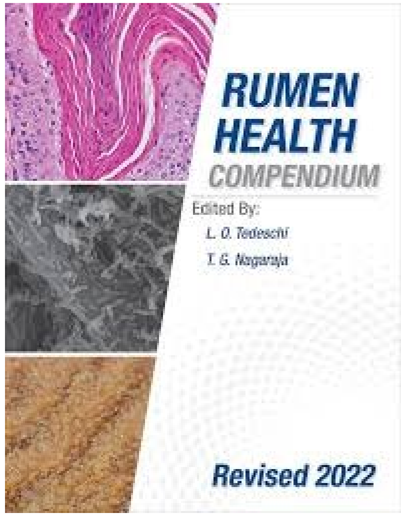
- Exposure to dietary mycotoxins adversely affects the performance and health of livestock.
  - We well know the effect of **Aflatoxins** and the problem of milk AFM1 contamination.
  - The recently published researches are clarifying some effects of **regulated *Fusarium* produced mycotoxins** on animal feeding behavior, productive and reproductive performance, as well as health status, immuno-metabolic issues and animal metabolism.
  - Very poor information are still available on emerging mycotoxins of silage (***Penicillium*, *Alternaria* and *A. fumigatus* produced mycotoxins**) and their effects on rumen and intestinal microbiota, as well as animal immunosuppression.

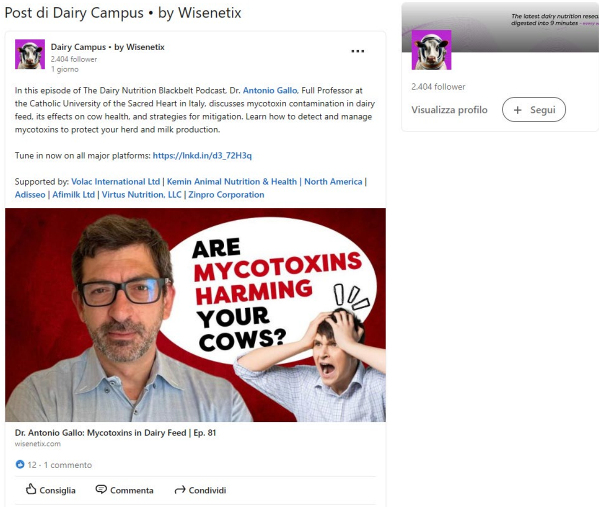
- Silage additives can be a promising alternative, other than best harvest and ensiling practices, to counteract negative effects of mycotoxin in livestock. Further studies are required to properly define which kinds of ***bacterial inoculants*** should be used, as well as ***chemical silage additives.***

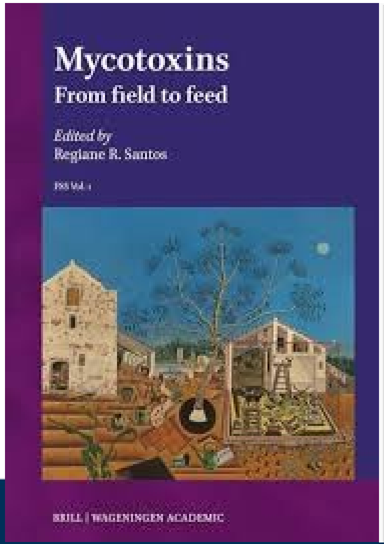
- To speed up the process of ***Silage Risk Assessment analysis***, simple, rapid, and accurate mycotoxin detection techniques should be developed and tested on field. The NIR is one of possible alternative.

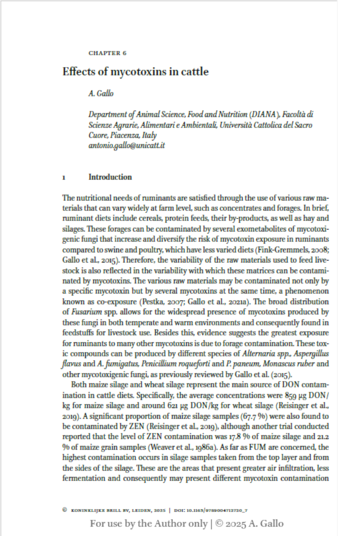
Recent Personal Contributions to Mycotoxin Research Field

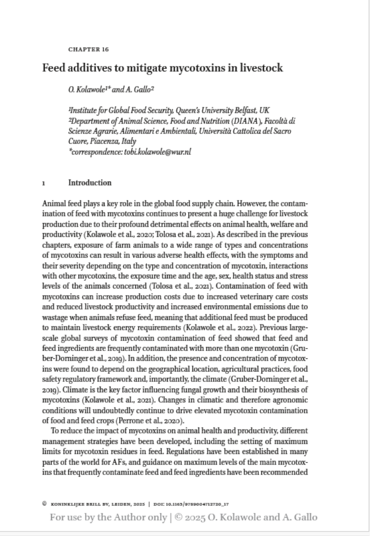












Two open questions for next ISC

Production of volatile organic compounds by mycotoxigenic fungi, can they be used as biomarkers?

- **VOCs** are products of the main fermentative pathways, and they can be used to evaluate the quality of ensiled forages (Hafner et al., 2013; Sigolo et al., 2023). **All fungi**, including mycotoxigenic species, **emit mixtures of VOCs** during the different phases of ensiling (Daniel et al., 2013; Weiss, 2017). The specific molecules and concentrations depend on the species of fungi and environmental conditions during growth (Bennet & Inamdar 2015).

Effects of mycotoxin on cattle, still we have to work on some regulated and emerging ones!?!

- **Livestock feeds** can be contaminated by several fungi. Among these, *Fusarium* spp. are widespread and produce mycotoxins, such as **deoxynivalenol (DON)**, **zearalenone (ZEN)**, and **fumonisins B1 and B2 (FB1 and FB2)**. In a recent paper (**Catellani et al. 2025. JDS https://doi.org/10.3168/jds.2025-26519**), we investigated whether feeding a TMR contaminated with moderate to high concentrations of ZEN, DON, FB1, and FB2 to dairy cows in early lactation alters:

  (1) their feeding behavior and rumination time;
  (2) milk yield, composition, and quality;
  (3) plasma metabolic profile and biochemical traits;
  (4) postpartum uterine involution and resumption of cyclicity.

Thanks to all people of my research group

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