Cow Nutrition School

University of Agriculture in Krakow

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UNIVERSITÀ CATTOLICA del Sacro Cuore

What are the effects of mycotoxins in dairy cattle?

March 12-13, 2025

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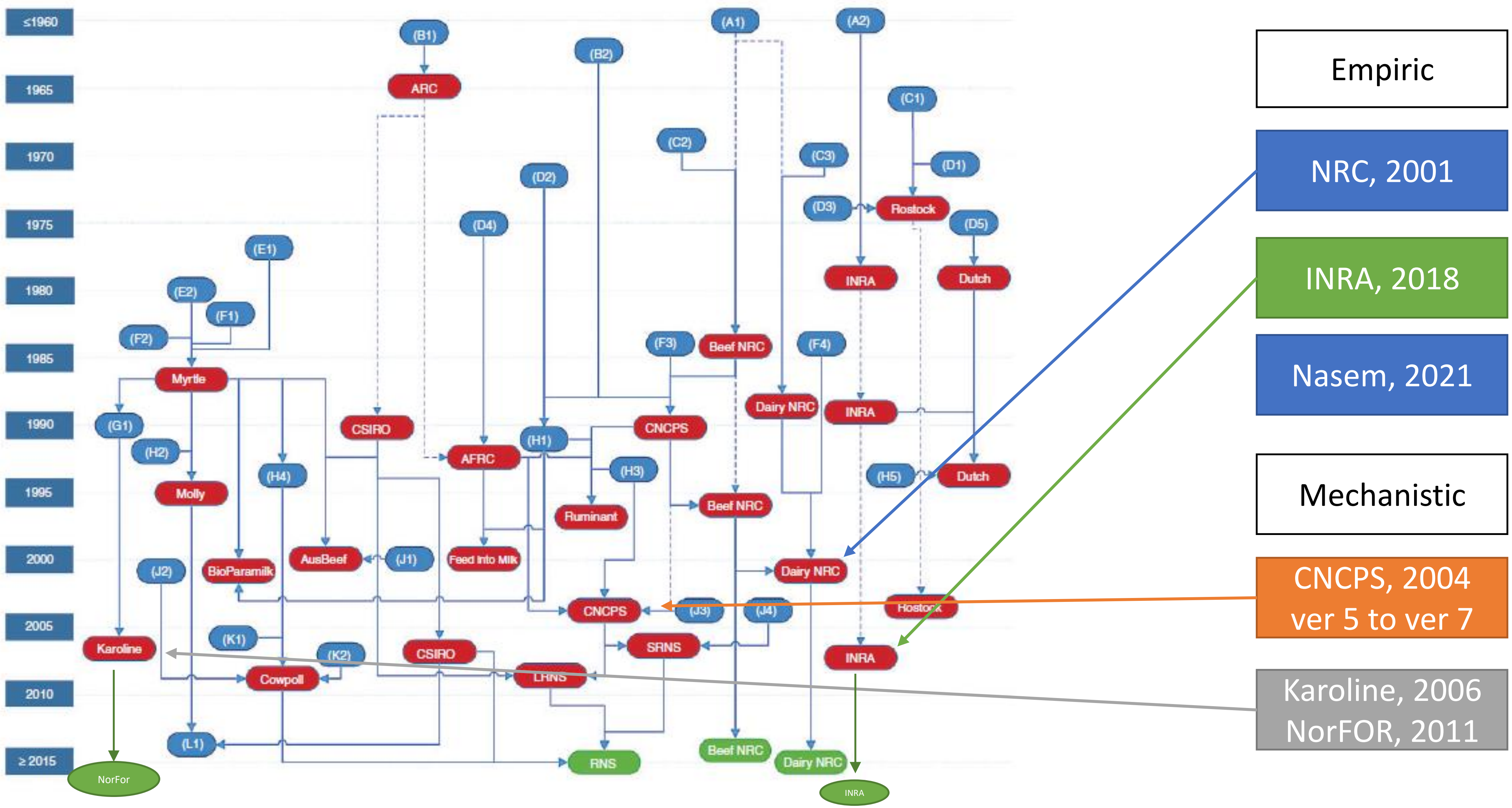
Piacenza-Cremona

RESTRICTED

Evolution of Ruminant Nutritional Models!!!



Tedeschi et al., 2014. The evolution and evaluation of dairy cattle models for predicting milk production: an agricultural model intercomparison and improvement project (AgMIP) for livestock. Animal Production Science, 54, 2052–2067

What About Mycotoxin in new Nasem 2021?!?

Chapter 17. Pages 342-343.

"Mycotoxin are naturally occurring toxins produced by molds"...

"Mycotoxin may be present in a variety of feeds provided to cattle, including **silages**, grains, pasture, hays, and **by-products feeds**, and can impair animal performance"...

"In addition to direct effects on the animal, some mycotoxins may have antibiotic properties than can affect rumen microbiota (Gallo et al., 2015) and so may have an indirect impact on performance"

NASEM 2021 contains approximately 250 mathematical formulas.

NONE has been published for MYCOTOXINS!!!!

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

Effect of Mycotoxins in animals

The term **mycotoxicosis** refers to the syndromes resulting from ingestion, skin contact or inhalation of these fungal metabolites.

When livestock ingest **one or more mycotoxins**, the effect on health could be **acute**, meaning evident signs of disease are present or even causing death. However, acute manifestation of mycotoxicosis is rare under farm conditions.

The effects of mycotoxin ingestion are mainly **chronic**, implying **hidden disorders with reduced ingestion, productivity and fertility**.

Such effects cause **severe economic losses** through **clinically ambiguous changes** in animal growth, feed intake reduction or feed refusal, alteration in nutrient absorption and metabolism, effects on the endocrine system as well as suppression of the immune system

Chronicle of Scientific interest on Mycotoxins

60s-70s

Aflatoxins B1, B2, G1, G2, M1

80s-90s up to now

Mycotoxins of cereals and other food for human consumption!

«Emerg … pp.

"Mycotoxins, wh … legislatively regulated. However, … rapidly increasing"

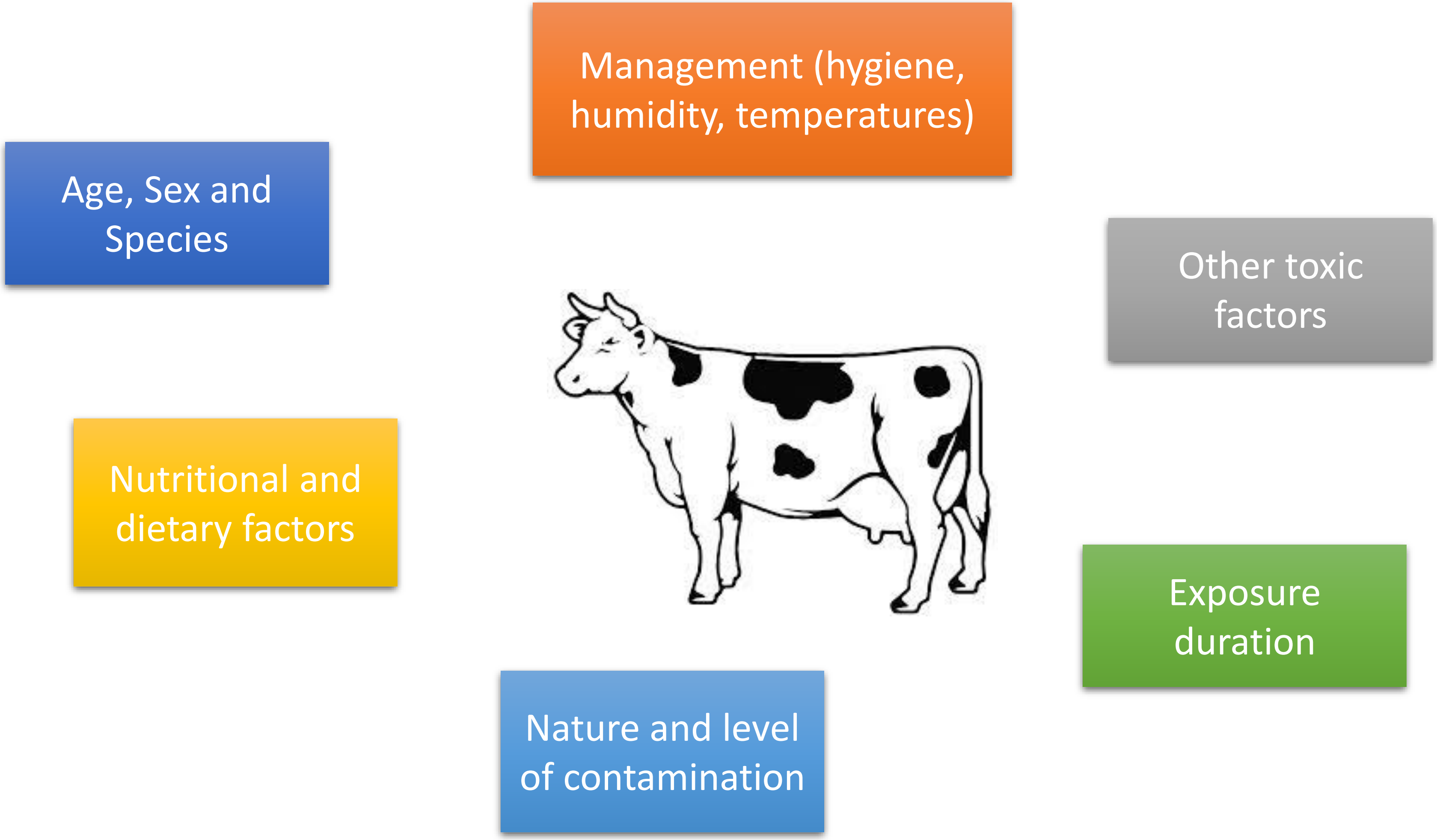
Bikaverina (BIK), Culmorin (CUL) Fusaric Acid (FA),

Beauvericin (BEA) & Enniatins (ENN), Moniliformin (MON),

Fusaproliferin (FUS), Sterigmatocystin (STE)

Review on Mycotoxin Issues in Ruminants

Gallo A, Giuberti G, Frisvad JC, Bertuzzi T, Nielsen KF. Toxins 2015, 7, 3057-3111.



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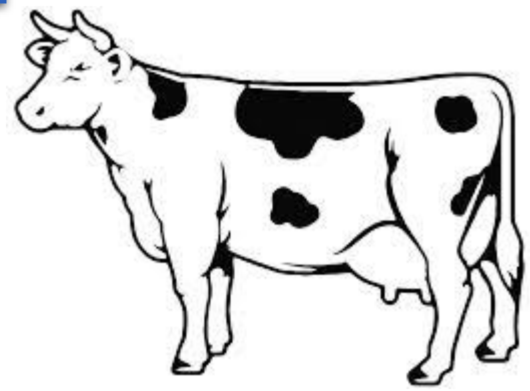
AFB1???
- Gastroenteritis
- Haemorrhagic intestine
- Reduces ruminal functionality
- Diarrhoea
- Ketosis

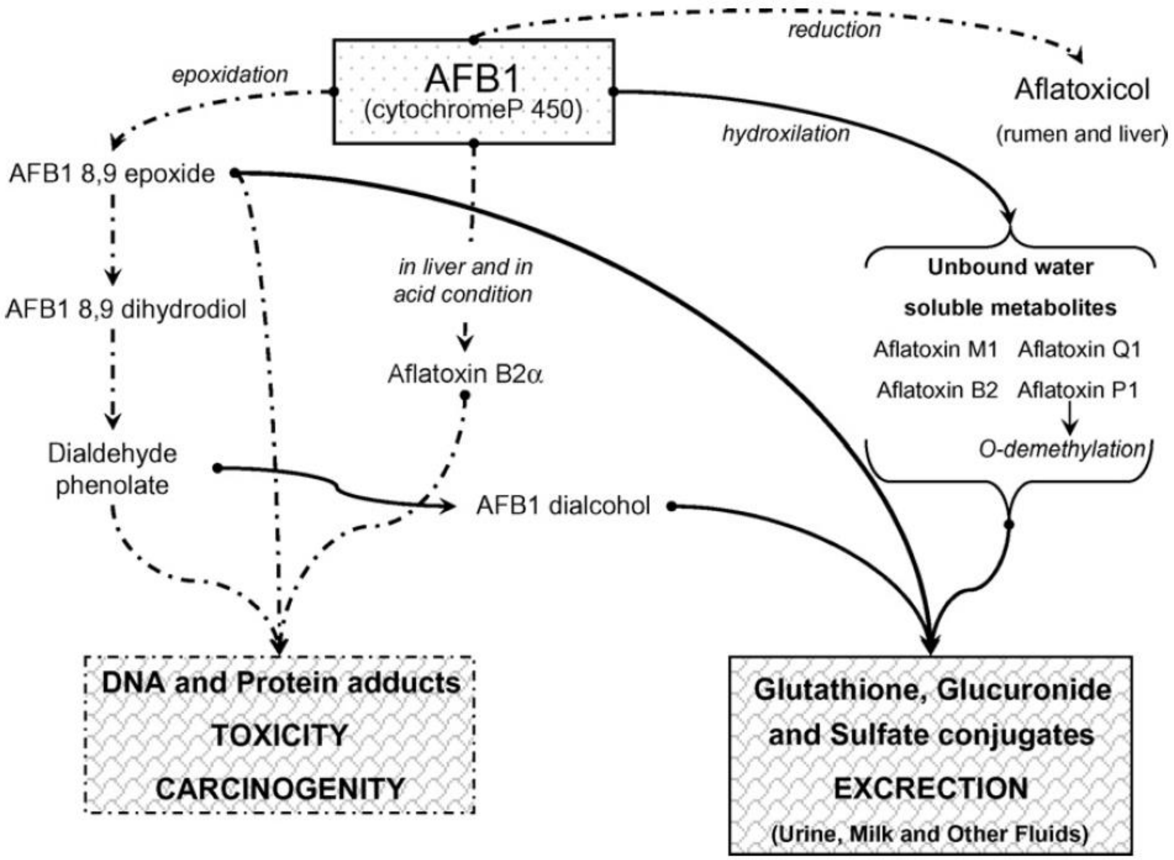


AFB1 toxic effects:
- Hepatotoxic, Hepatocarginogenic
- Neurotoxic, Nephrotoxic
- Homotoxic, Enterotoxic
- 0steotoxic, Immunosuppressive

AFB1
- Metabolites in milk
- Reduces milk production
- Mastitis???

Aflatoxin Metabolism

Can be absorbed by Ingestion, Contact and Inhalation



Excretion mechanism

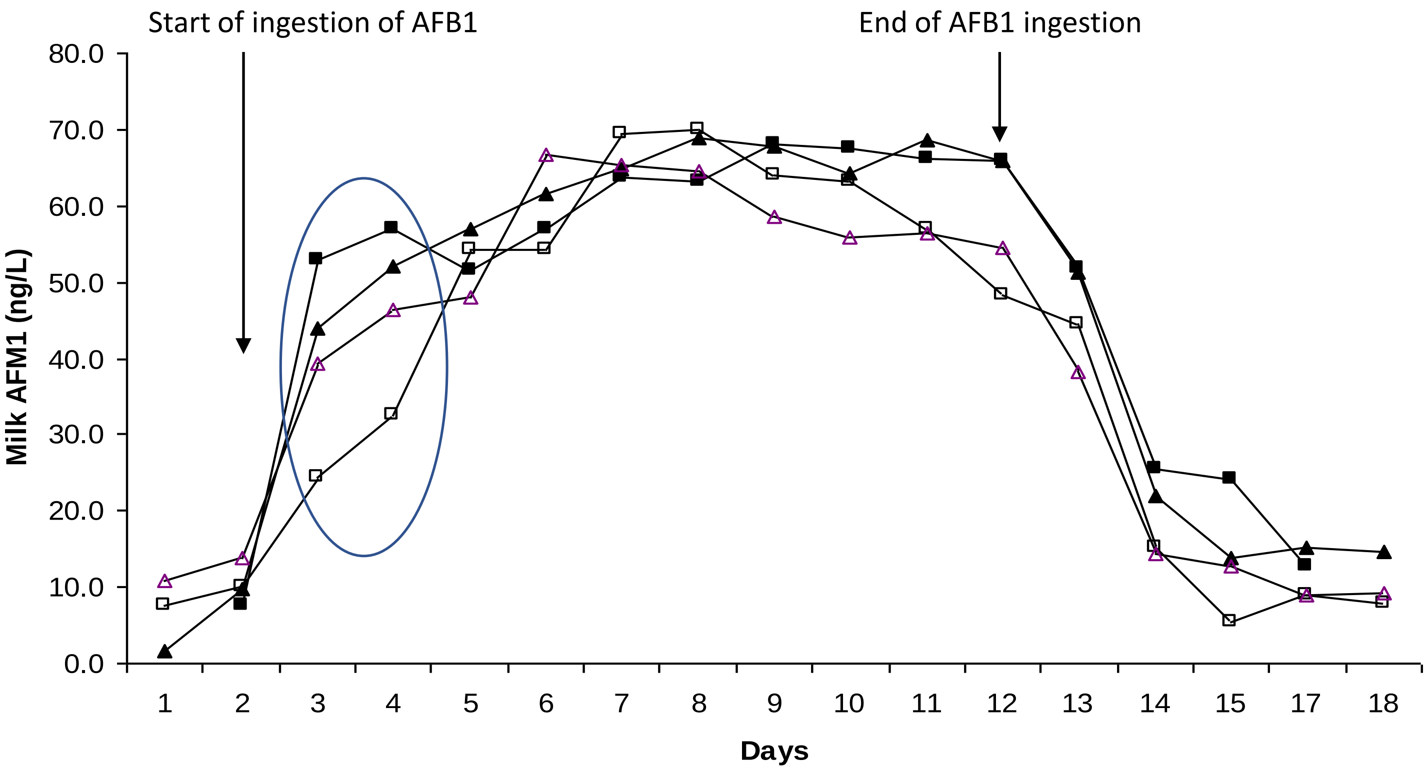
- Faeces : 55-80%
- Urine : 10-20%
- Meat : <1%
- Milk : 1-3%

Moschini et al., AFST, 2009

Effect of SCCs on AFM1 in milk

Trend of AFM1 concentration (ng/L) in cows analysed in the experimental design (■) HYHSCC, (□) HYLSCC, (▲) LYHSCC, (Δ) LYLSCC

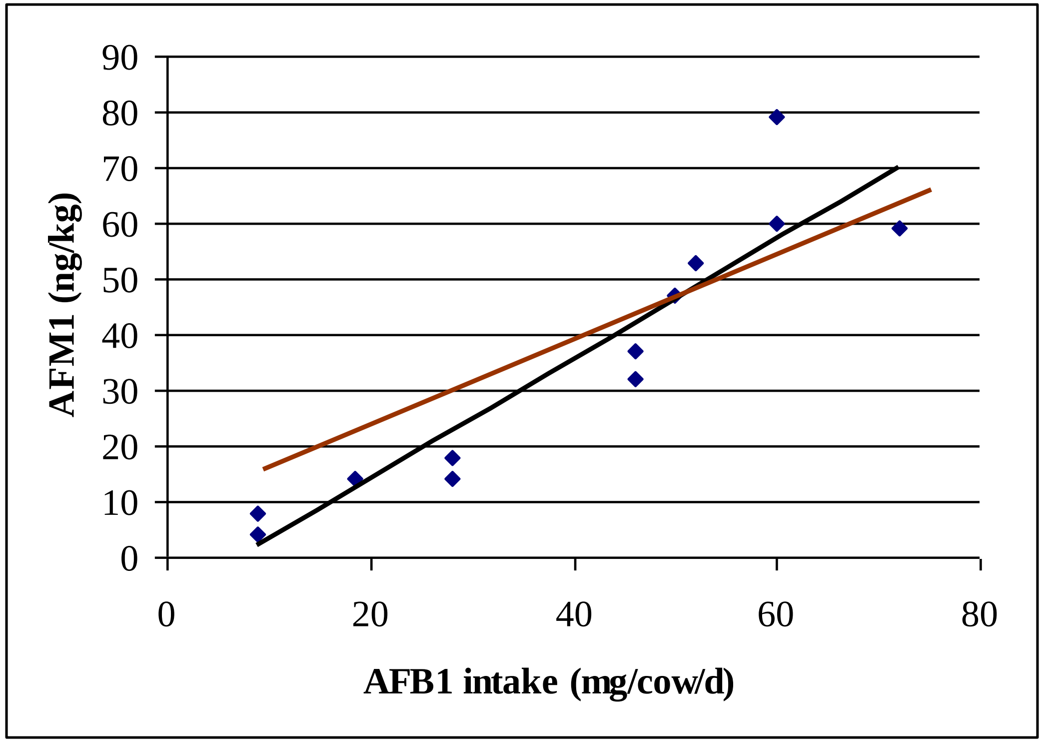
Cows assumed 90 µg/head/day fixed



Masoero et al., Animal 2007

Effect of SCCs on AFM1 in milk

- AFM1 (ppt) = 1.19 x AFB1 (µg/vacca/d) + 1.9 (Veldman et al. 1992)
- AFM1 (ppt) = 0.787 x AFB1 (µg/cow/d) + 10.95



Review on Mycotoxin Issues in Ruminants

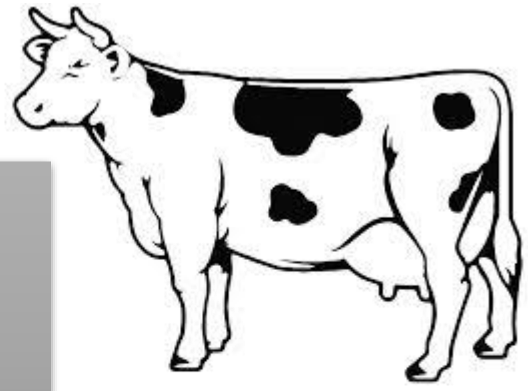
Gallo A, Giuberti G, Frisvad JC, Bertuzzi T, Nielsen KF. Toxins 2015, 7, 3057-3111.

DON, T-2 toxin
- Gastroenteritis
- Hemorrhagic bowel
- Reduces ruminal function and intestinal absorption
- Diarrhoea
- Ketosis

Boguhn et al., 2010; Hildebrand et al., 2012; Jeong et al., 2010; Keese et al., 2008a; Dänicke et al., 2005

DON, T-2 toxin, DAS, FBs
- Feed Refusal
- Reduced DMI
- Reduced Feed efficiency

Trenholm et al., 1985; Kiyothong et al., 2012, Harvey et al., 1995



DON
- Lameness
- Immunosuppression

Korosteleva et al., 2007; 2009

ZEA
- Irregular heats
- Reduces CR
- Ovarian Cysts
- Embryo loss
- Abortions
- Reduced testicular development
- Reduced spermatogenesis

Weaver et al., 1986; Coppock et al., 1990; Smith et al., 1990

DON, FBs
- Hepatic alterations

Osweiler et al., 1993; Baker et al., 1999; Hochsteiner et al., 2000; Abeni et al., 2014;

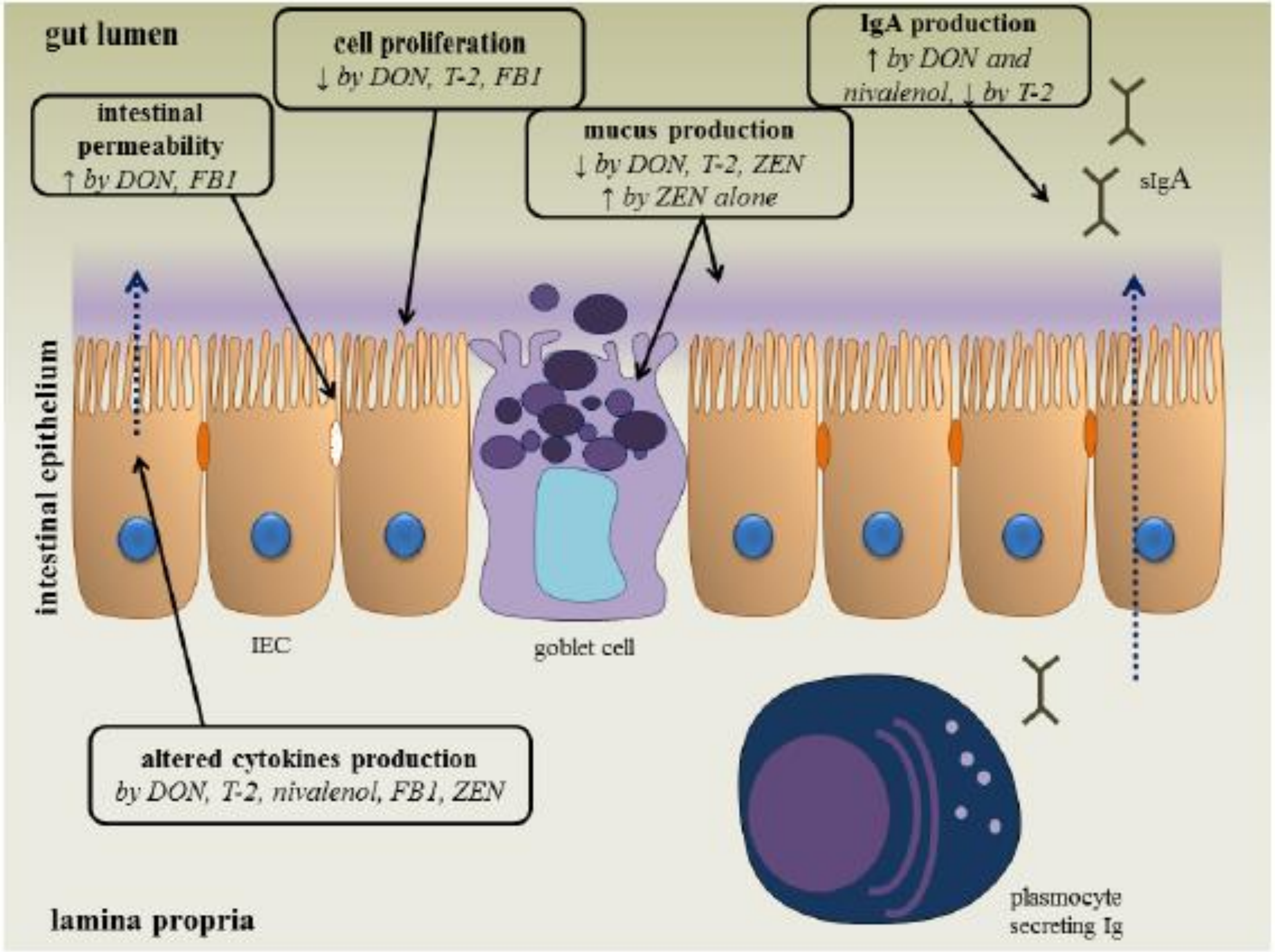
DON, T-2 toxin
- Reduces milk production
- Mastitis

T2&HT2 → trials between 70s-80s and on young animals. No info on adult cattle (EFSA, 2011)

Nivalenol, Fusarenon X → No Info (EFSA 2013)

Beauvericin, Enniatins, Moniliformin → No Info

The effect of *Fusarium* mycotoxins on the intestinal epithelium

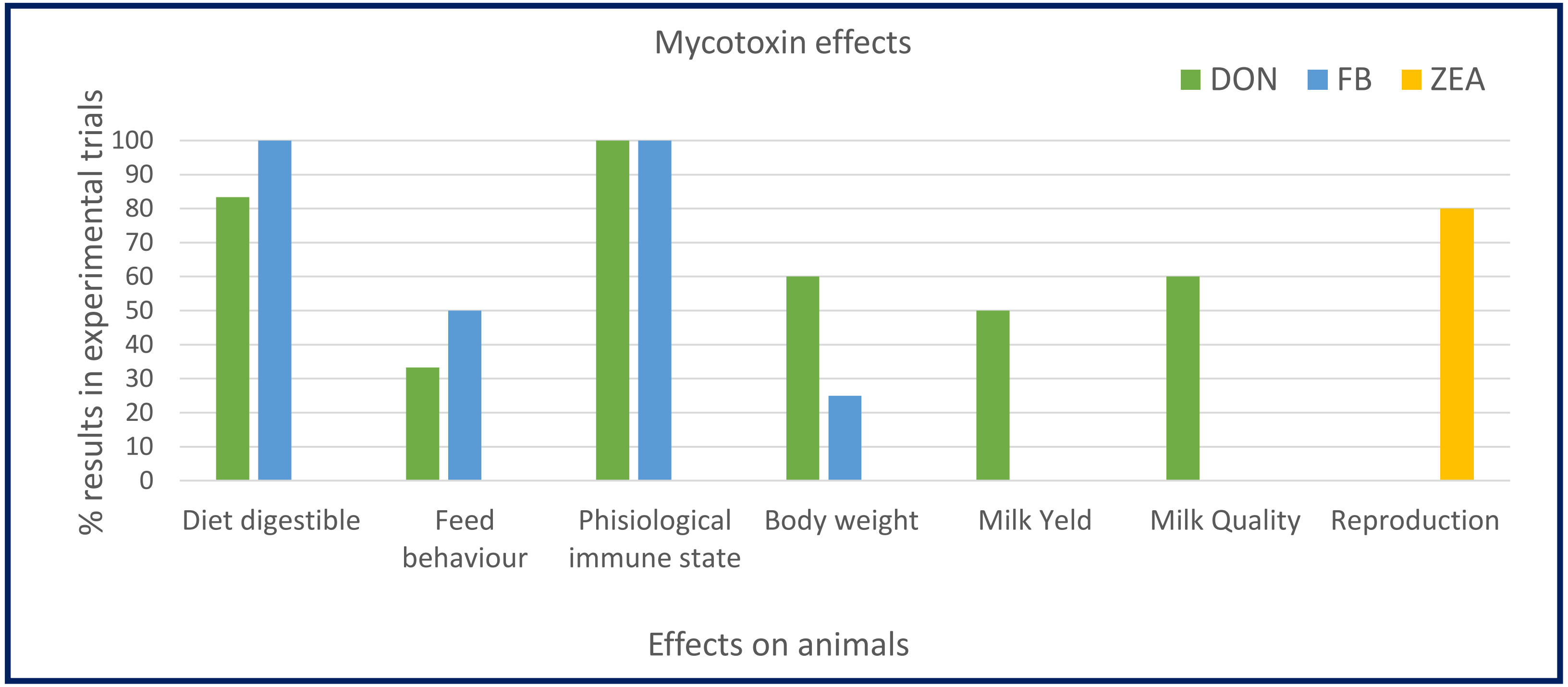


Reproduced from **Antonnisen et al.**, **2014**.

Adverse Effects of Fusarium Toxins in Ruminants: A Review of in vivo and in vitro Studies

Gallo A, Mosconi M, Trevisi E, Santos R. 2022. Dairy 2022, 3, 474–499.

Scientific articles published in the last 7 years



- **Summary of 21 scientific trials**
- **Effect of main *Fusarium* mycotoxins (i.e., DON, ZEN, FB)**
- **When mycotoxins with different effects were studied in the same trial, each effect was attributed only to the mycotoxin responsible for it**

Bibliography used: Duringer J.M. et al; 2020; World Myco. J.- Roberts H. L. et al.; 2021; Toxins- Gallo A. et al; 2020; J. Dairy Sci.- Danicke S. et al.; 2016; Arch. Anim. Nutrit. - Jovaisiene J. et al.; 2016; Pol. Jour. Vet. S. - Kinoshita A. et al.; 2015; J. of Phys. and Anim. Nutr.- Jennings J.S. et al.; 2020; J. Anim. Sci. - Fushimi Y. et al.; 2015; Reprod Dom Anim. - Almeida Silva L. et al.; 2021; Reprod. Dom. Anim. - McKay et al., (2019); Anim. Feed Sci. Technol. - Hildebrand B. et al; 2012; J. Anim. Physiol. Anim. Nutr. - Keese, C.; 2008; Arch. Anim. Nutr. - Keese, C.; 2008; Arch. Anim. Nutr. - Korosteleva, S.N.; 2007; J. Dairy Sci. - Ingalls, J.R.; 1996; Anim. Feed Sci. Technol. - Weaver, G.A.; 1986; Am. J. Vet. Res. - Coppock, R.W.; 1990; Vet. Hum. Toxicol. - Baker, D.C.; 1999; J. Vet. Diagn. Investig.- Osweiler, G.D.; 1993; J. Anim. Sci. - Mathur, S.; 2001; Toxicol. Sci.- Weaver, G.A.; 1986; Am. J. Vet. Res.

A mycotoxin-deactivating feed additive counteracts the adverse effects of regular levels of Fusarium mycotoxins in dairy cows.

Gallo et al. 2020. Journal of Dairy Science 103, 11314-11331

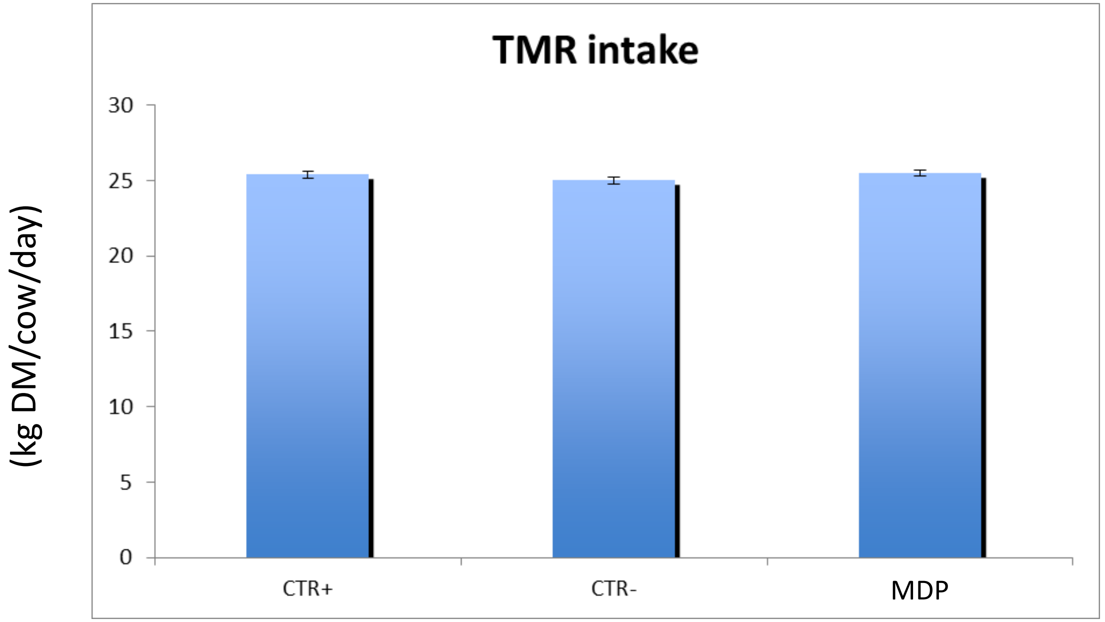
Experimental periods in 3 x 3 Latin Square Design
Adaptation (14gg)
Spring Intoxication period (21gg)
Wash out (14gg)
Second intoxication period (21gg)
Wash out (14gg)
Third intoxication period (21gg)

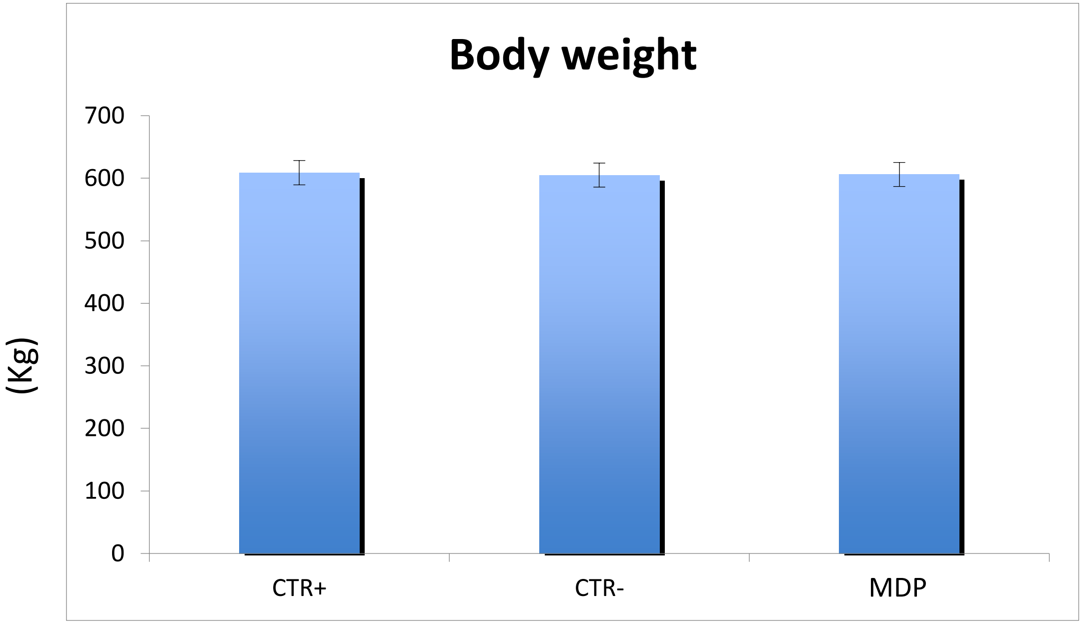
Contamination of diets			
Mycotoxins (µg/kg DM)	Control (CTR+)	Contaminated Diet (CTR-)	Contaminated Diet + MDP (MDP)
AFB1	0.057	0.445	
DON	447	1’061 (x 2-3 times)	
ZEA	7	37	
FB1+FB2	117	1’050 (x 10 times)	
HT-2	2	8	
T-2	6	31	

Animals	Latin Square	Period 1	Period 2	Period 3
Cow 1	Low MY	CTR-	CTR+	MDP
Cow 2	Low MY	MDP	CTR-	CTR+
Cow 3	Low MY	CTR+	MDP	CTR-
Cow 4	Medium MY	MDP	CTR+	CTR-
Cow 5	Medium MY	CTR+	CTR-	MDP
Cow 6	Medium MY	CTR-	MDP	CTR+
Cow 7	Medium MY	CTR-	MDP	CTR+
Cow 8	Medium MY	MDP	CTR+	CTR-
Cow 9	Medium MY	CTR+	CTR-	MDP
Cow 10	High MY	CTR+	CTR-	MDP
Cow 11	High MY	MDP	CTR+	CTR-
Cow 12	High MY	CTR-	MDP	CTR+

A mycotoxin-deactivating feed additive counteracts the adverse effects of regular levels of Fusarium mycotoxins in dairy cows.

- No **BCS** variation during the trial
- No **Dry Matter Intake** variation (25.3 kg/capo/giorno)
- No **Body weight** variation (606 kg)



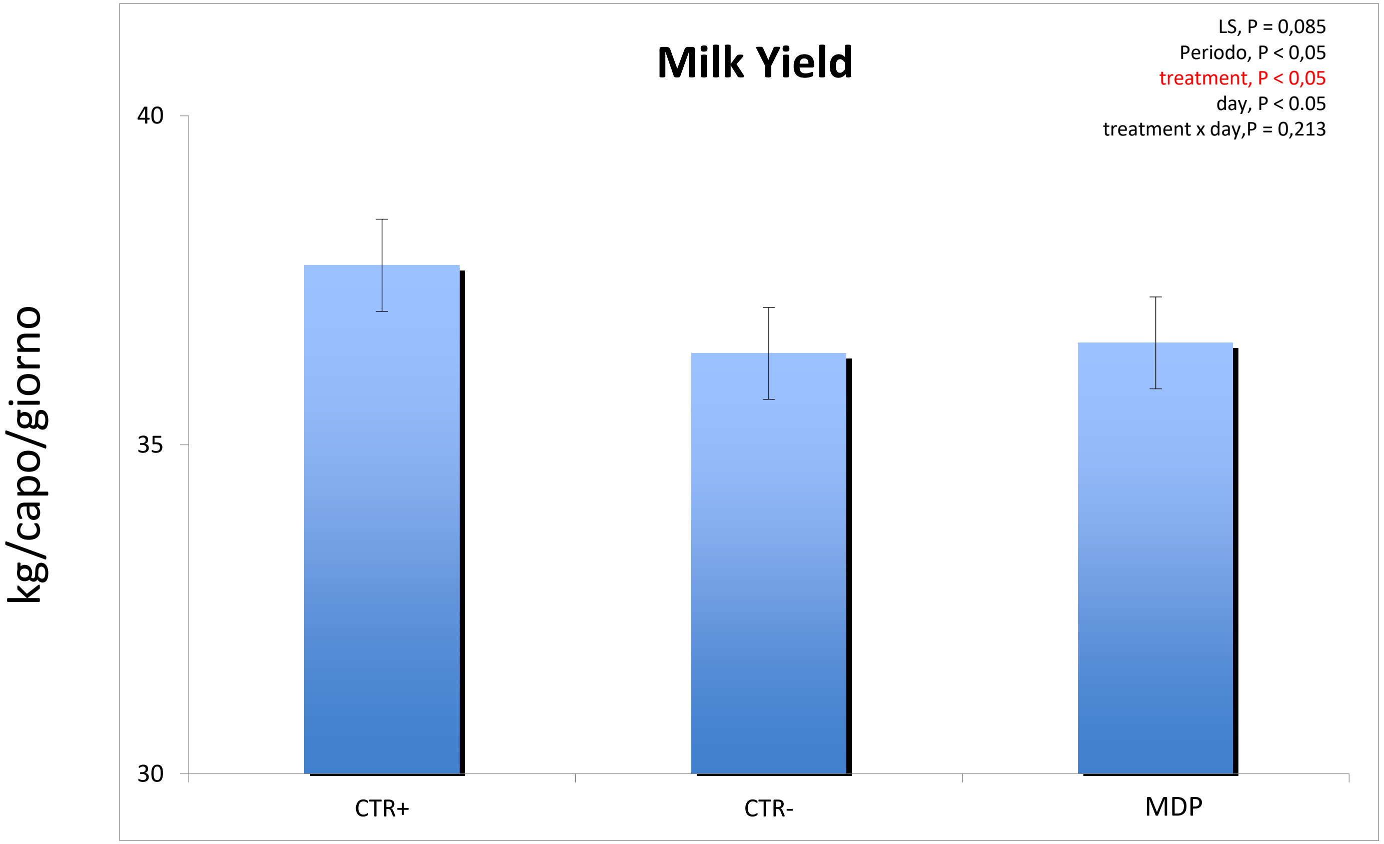


A mycotoxin-deactivating feed additive counteracts the adverse effects of regular levels of Fusarium mycotoxins in dairy cows.

The Milk yield was reduced by

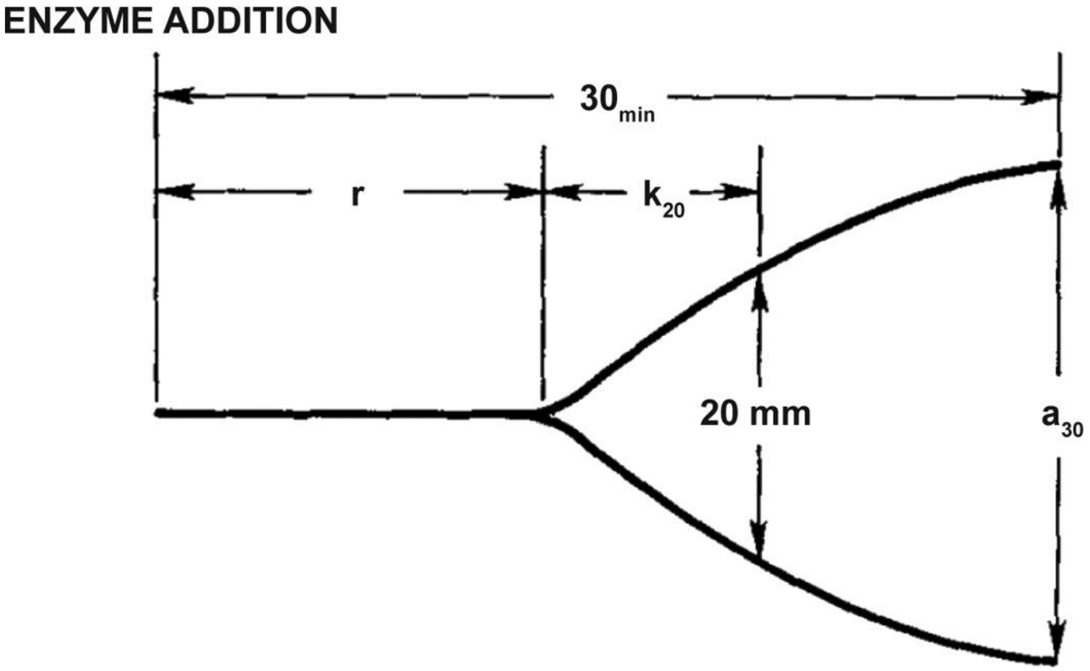
-1,34 kg/cow/day for **CTR- vs. CTR+**

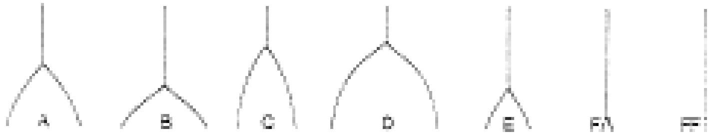
-1,14 kg/cow/day for **MDP vs. CTR+**



A mycotoxin-deactivating feed additive counteracts the adverse effects of regular levels of Fusarium mycotoxins in dairy cows.

Diagram of **rennet coagulation time** (r, min), **curd firmness traits** (k_{20}, min), and **curd firmness 30 min after enzyme addition** (a_{30}, mm) as a function of time (lactodynamographic curve, Formagraph Foss Electric A/S, Hillerød, Denmark).
Reproduced by Bittante et al. 2012.





A - Milk with **good coagulation** characteristics

B - Milk with a **long coagulation time** but **good coagulation** setting speed, relatively **high final consistency**. This usually occurs with milk from the **end of lactation and/or rich in casein**

C - Milk with a **short coagulation time**, but **low coagulation** setting speed, relatively **poor final consistency**. It is usually present with relatively **poor casein** milk especially **at the beginning of lactation**

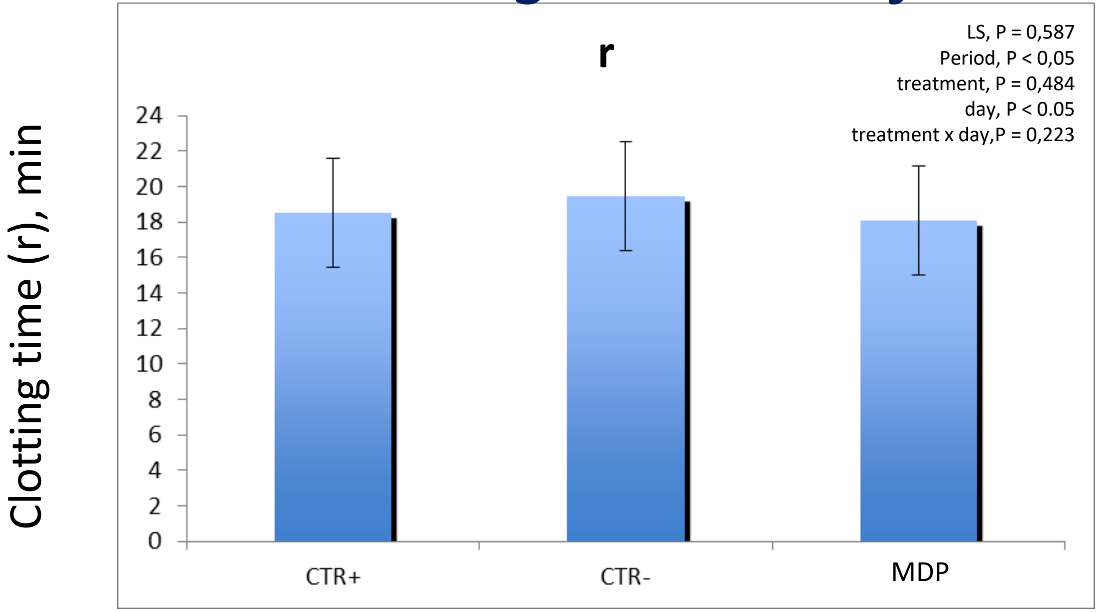
D - Milk with **short coagulation time**, **high setting speed** and **excessive final consistency**. Typical tracing of mature milk or milk from **fresh farrowing cows;** sometimes milk from **traditional breeds of cattle** (Bruna Alpina, Reggiana) can have such a tracing even under normal conditions

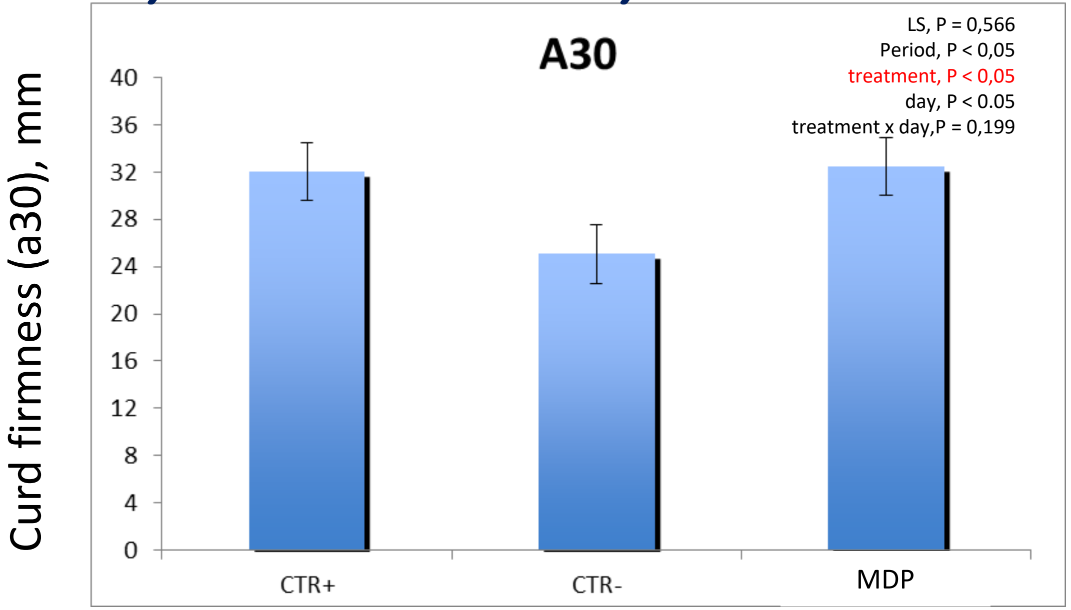
E - Milk with **long coagulation time**, **low setting speed** and **poor final consistency**. It is caused by genetic predisposition, **hypoacidity, mastitis, milk of too long lactation, strong environmental stress,** nutritional errors, **pathologies in progress**

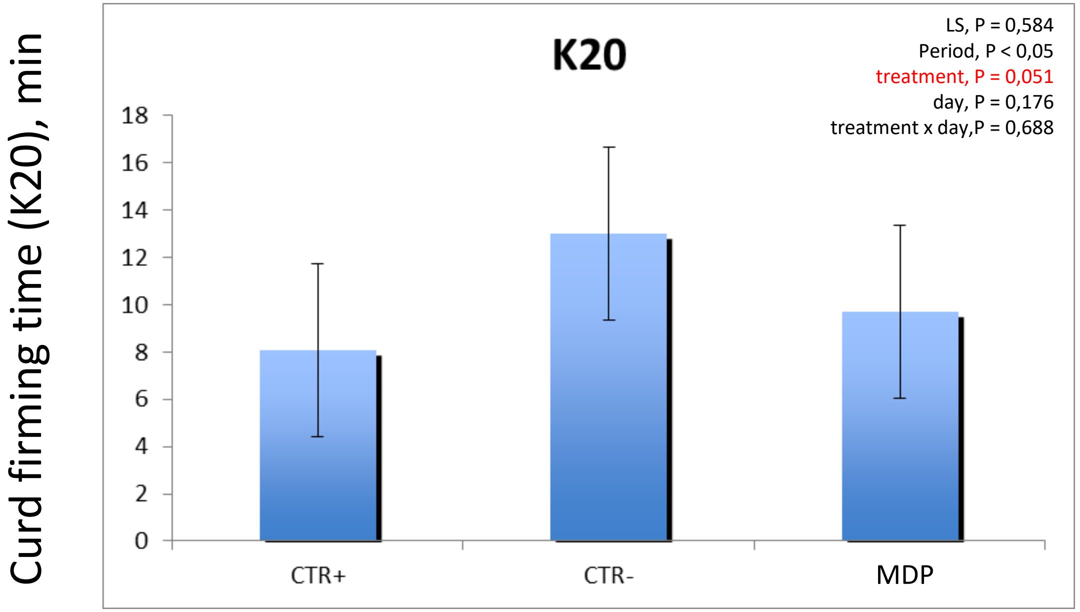
F - Milk with **very long coagulation time**, **very low setting speed** and very **little final consistency**

FF- Milk that **does not coagulate** during the technical times of the lactodynamic test. As the F and FF types are worsening of E, the predisposing causes are the same.

A mycotoxin-deactivating feed additive counteracts the adverse effects of regular levels of Fusarium mycotoxins in dairy cows.







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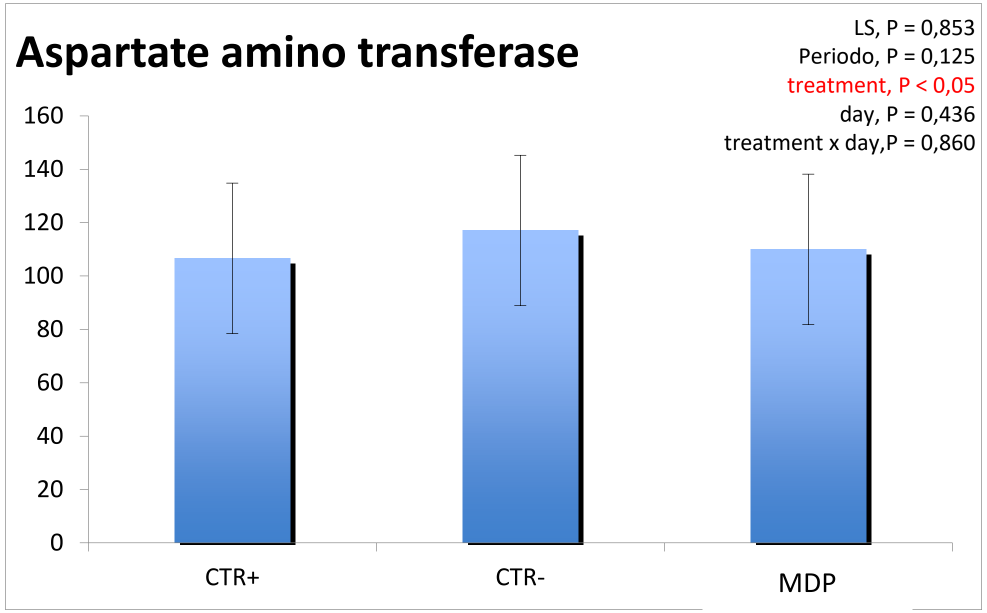
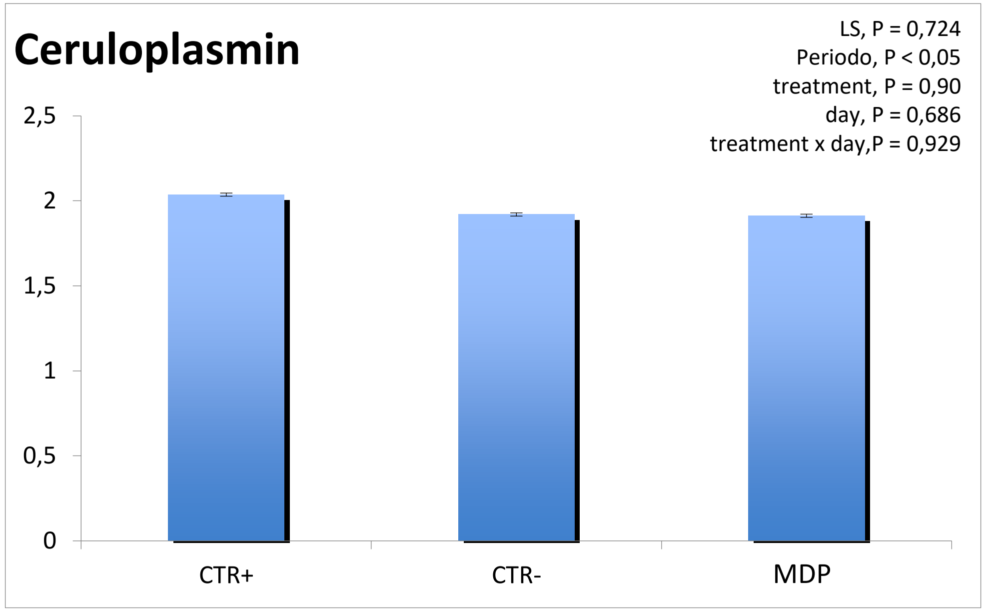
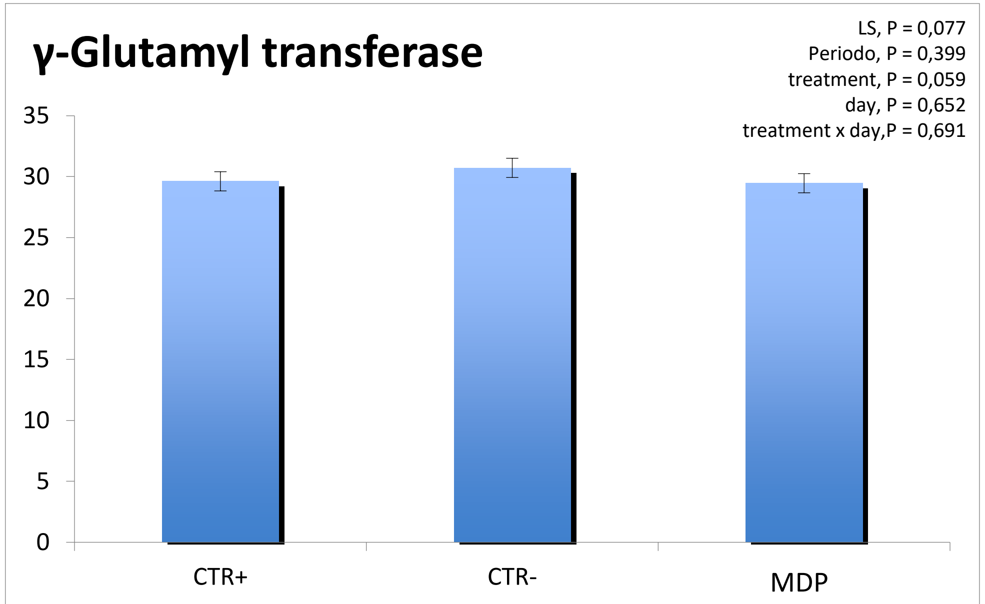
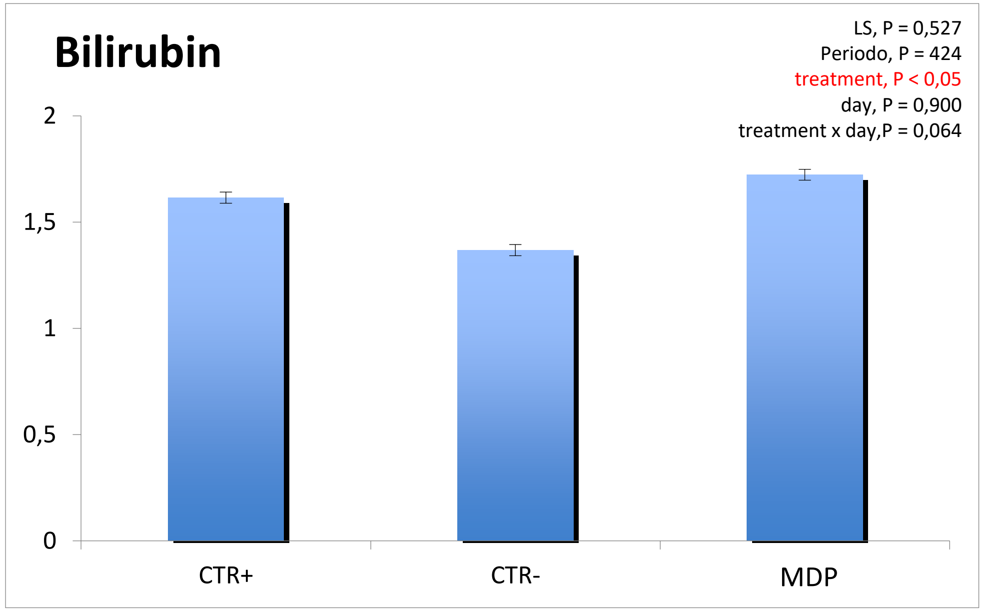


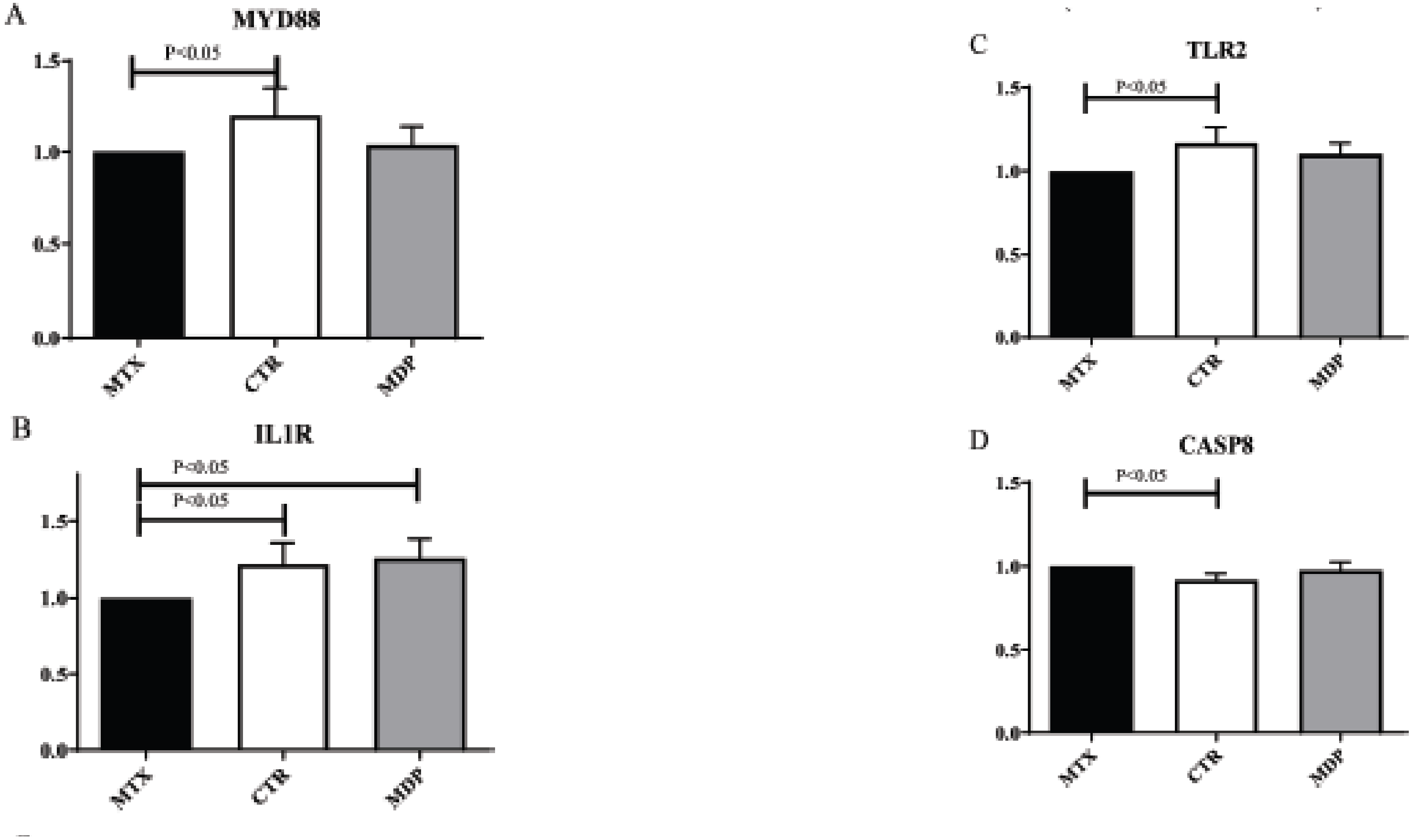






A mycotoxin-deactivating feed additive counteracts the adverse effects of regular levels of Fusarium mycotoxins in dairy cows.

The MTX diet altered the expression of several genes in circulating leucocytes. In particular, was observed **a lower expression of genes** that are important mediators of **immune and inflammatory responses** (*MYD88*, *IL1R*, and *TLR2*). Their lower expression could be because **the *Fusarium* mycotoxins had an immunosuppressive effect.** This interpretation is supported by the higher expression of the CASP8 gene in the MTX group



Effects of a mycotoxin mitigation feed additive in lactating dairy cows fed *Fusarium* mycotoxin-contaminated diet for an extended period

Cattelani et al., 2023. Toxins, 15(9), 546.

- Little is known about the adverse effects of commonly found levels of *Fusarium* mycotoxins on dairy cow performance, **especially after a long period of exposure (54 days)**.
- To study the effects of moderate levels of **Deoxynivalenol** (DON), **Zearalenone** (ZEA) and **Fumonisin B1 & B2** (FB) from feeds naturally contaminated
- 36 lactating Holstein cows were used in a completely randomized design.

Experimental periods
Adpatation (7 gg)
Spring intoxication period (54 gg) – 18 cows
Wash out (7 gg)
Summer intoxication period (54 gg) – 18 cows
Wash out (7 gg)

Diet mycotoxins contamination			
Micotoxins (µg/kg DM)	CTR	MTX	MMP
AFB1	nd	nd	
DON	284	1’020 (x2-3 volte)	
ZEA	43	230	
FB1+FB2	117	1’054 (x10 volte)	
HT-2	4	9	
T-2	5	11	

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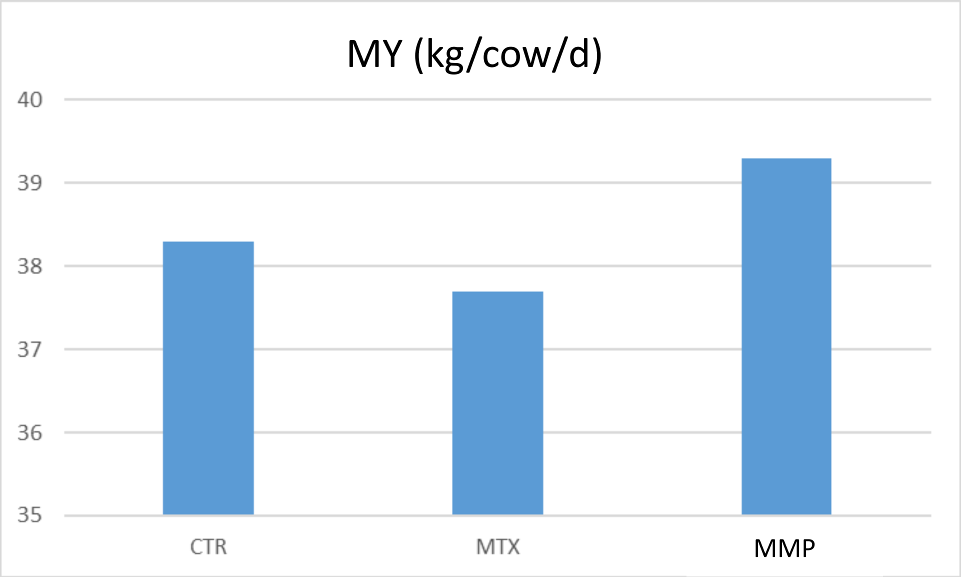
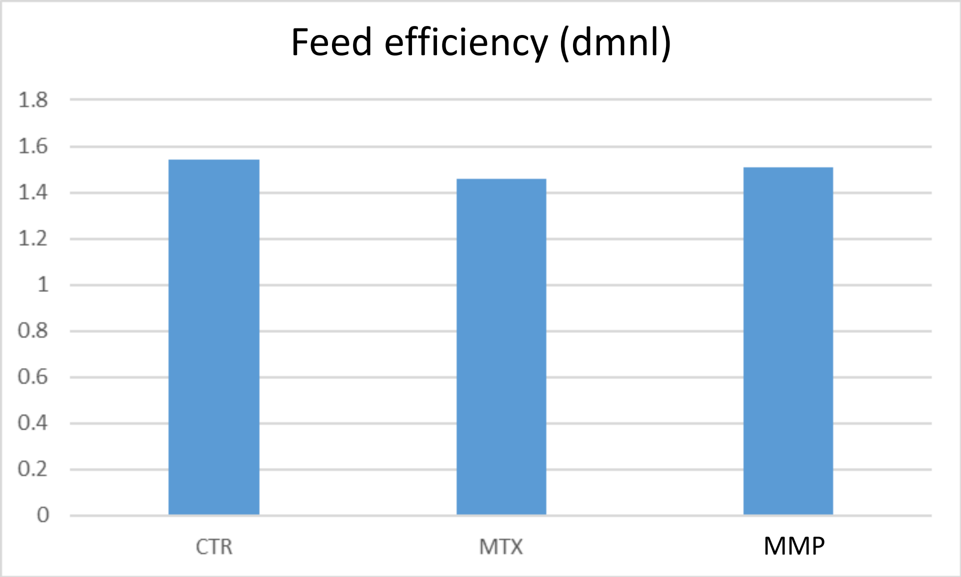




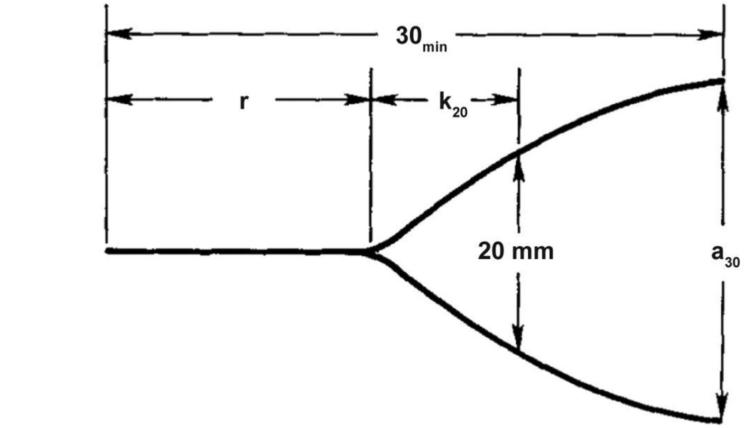
Anyway, no differences for:

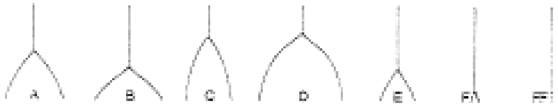
- Fat of milk (% or kg/d)
- Crude Protein of milk (% or kg/d)
- Lactose of milk (% or kg/d)
- Casein of milk (% or kg/d)
- MUN (mg/100 ml)
- SCC (Log_{10}cells/mL)

Effects of a mycotoxin mitigation feed additive in lactating dairy cows fed Fusarium mycotoxin-contaminated diet for an extended period

Milk coagulation properties:

Diagram of **rennet coagulation time** (r, min), **curd firmness traits** (k_{20}, min), and **curd firmness 30 min after enzyme addition** (a_{30}, mm) as a function of time (lactodynamographic curve, Formagraph Foss Electric A/S, Hillerød, Denmark). Reproduced by Bittante et al. (2012) & Cecchinato et al. (2015)





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F - Milk with **very long coagulation time, very low setting speed** and very **little final consistency**

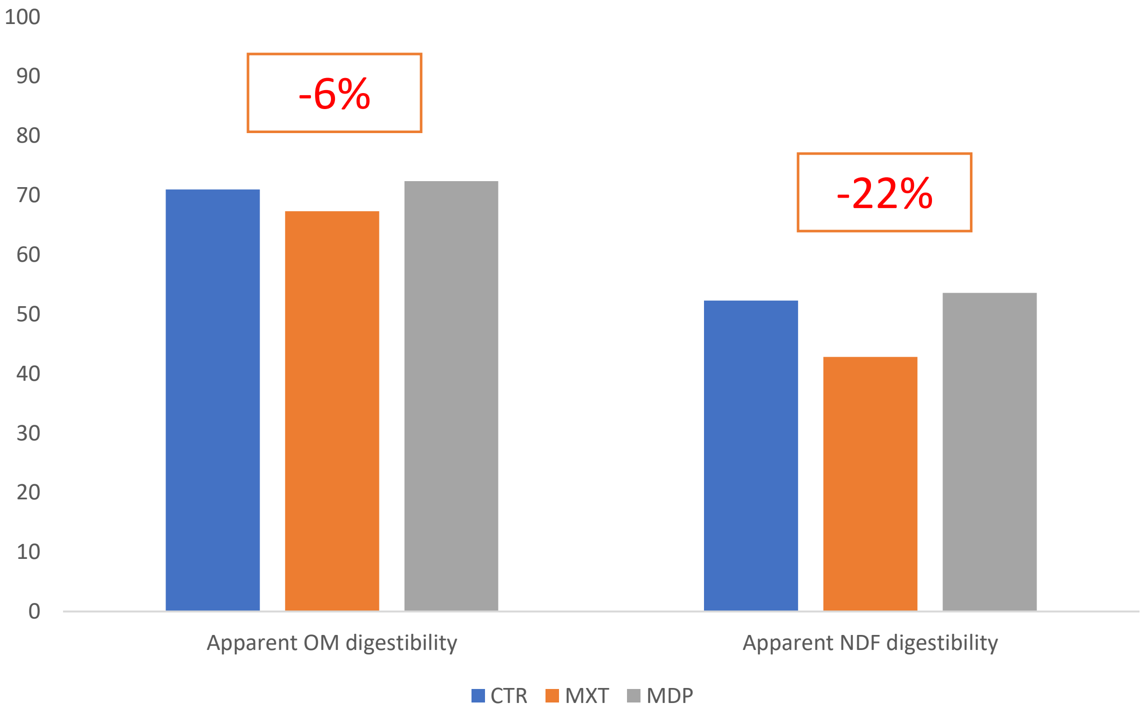
FF- Milk that **does not coagulate** during the technical times of the lactodynamic test. As the F and FF types are worsening of E, the predisposing causes are the same.

Items		Treatments			Periods		sem	P <			
		CTR	MTX	MMP	Spring	Summer		Period	Treatment (T)	Week (W)	W * T
Casein index	%	79.0	78.4	79.7	80.4	78.2	0.829	<0.05	0.208	<0.05	0.298
r	min	23.9	29.0	24.1	25.9	24.9	6.081	0.739	0.547	0.164	0.541
A_{30}	mm	18.99	12.27	18.78	17.82	16.60	11.302	0.795	0.675	0.775	0.571
K_{20}	min	7.98	10.50	8.21	9.26	8.46	1.299	0.653	0.738	0.157	0.087

Apparent TT digestibility of main nutrients

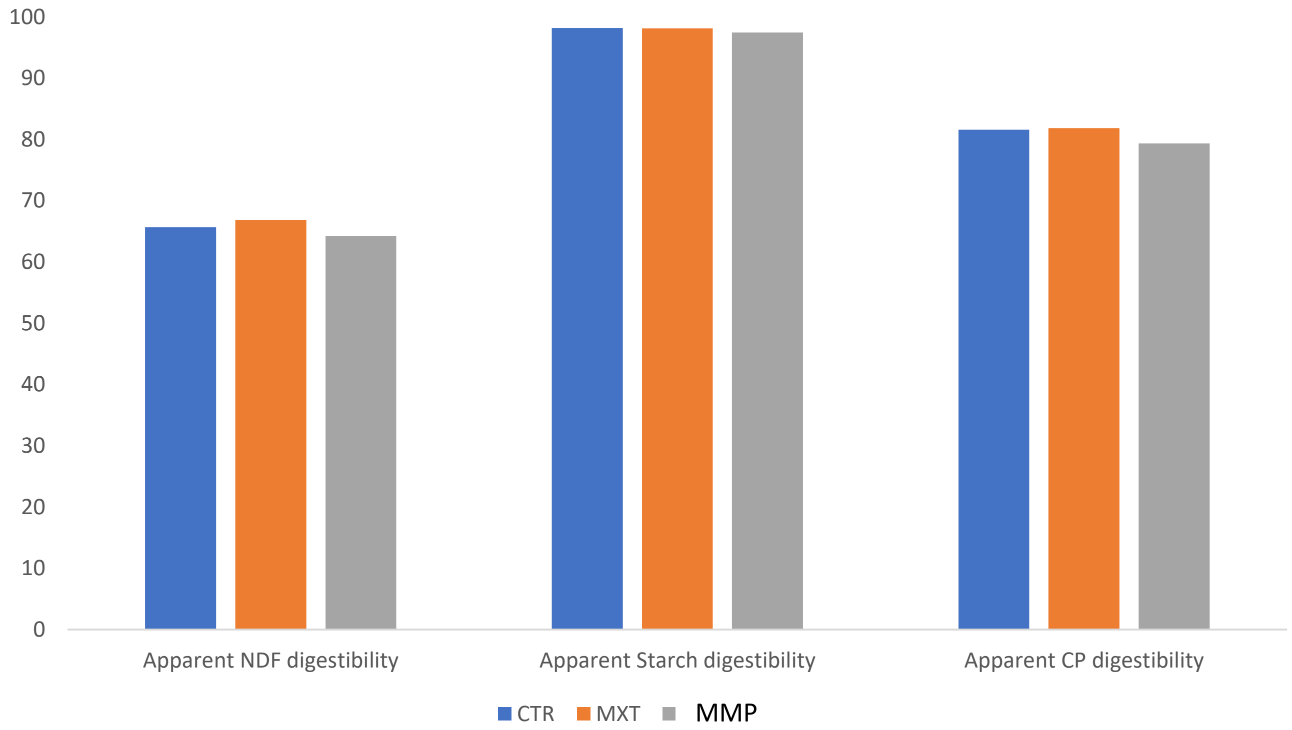
A mycotoxin-deactivating feed additive counteracts the adverse effects of regular levels of Fusarium mycotoxins in dairy cows.

Gallo et al. 2020. Journal of Dairy Science 103, 11314-11331



Effects of a mycotoxin mitigation feed additive in lactating dairy cows fed Fusarium mycotoxin-contaminated diet for an extended period

Gallo A et al. 2023 Toxins, under review



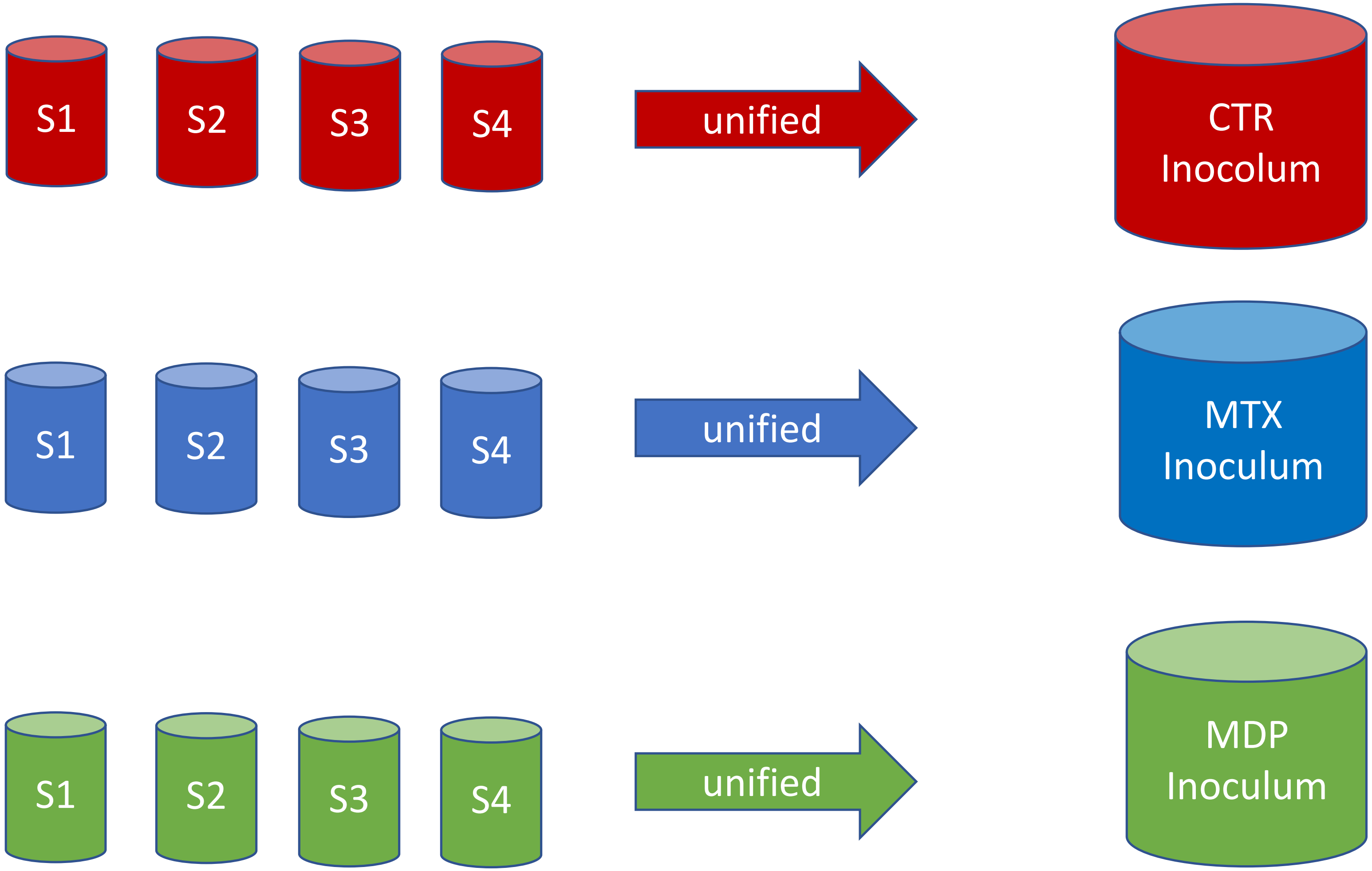
Kinetics of gas production in the presence of Fusarium mycotoxins in rumen fluid of lactating dairy cows.

Gallo A. 2021. JDS Communication 2, 2021; 2:243–247

For each experimental period
of 3 x 3 Latin Square Design
Details in Gallo et al. 2020 (J Dairy Sci. 103.
https://doi.org/10.3168/jds.2020-18197)

Rumen samples collected via esophageal tubing
12 rumen fluids for each period

Creation of Rumen *inocula* for in vitro gas production test
(CTR, MTX, MDP) for each run



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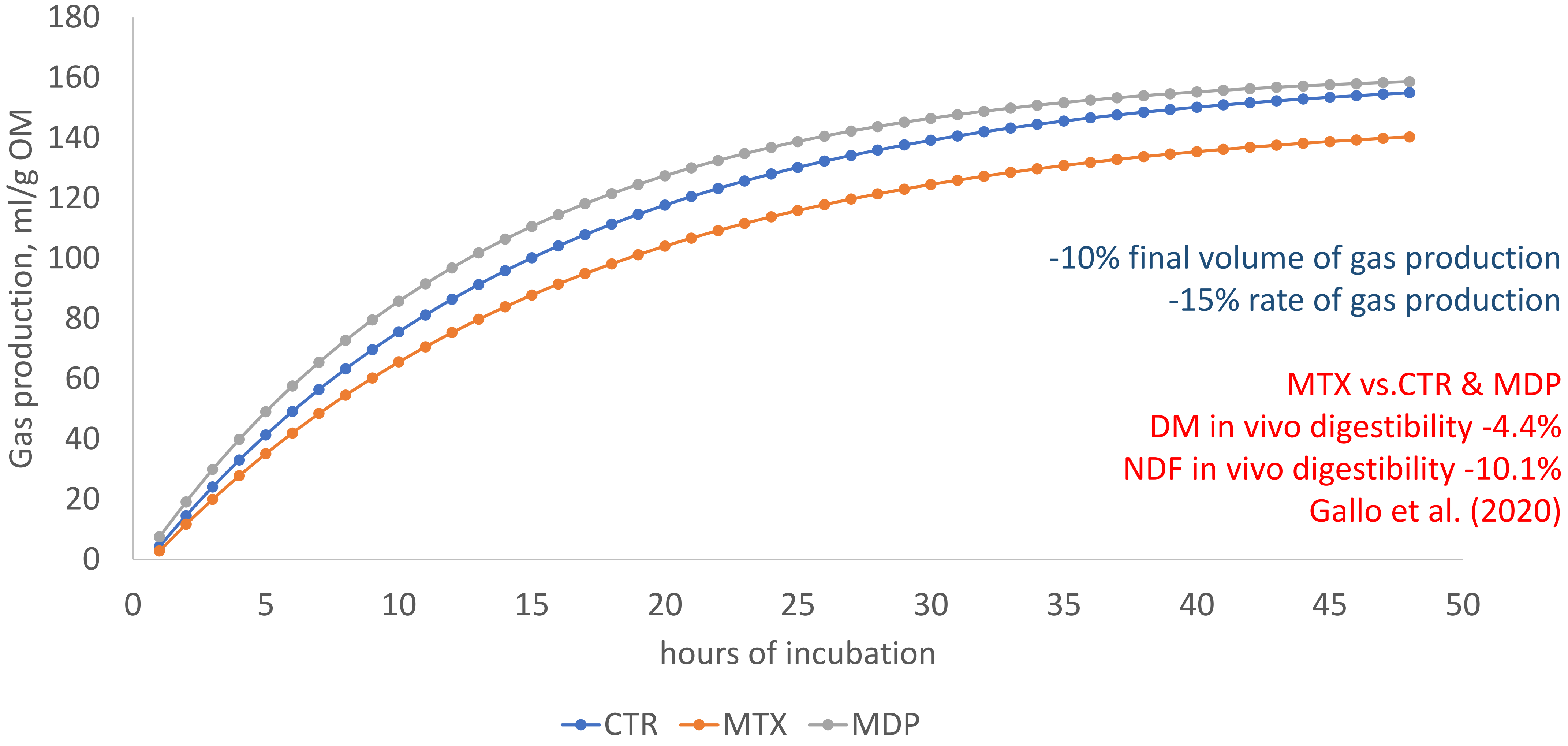


Mycotoxins and Ruminants

RUMINANTS less susceptible than MONOGASTRICS

Rumen represents an active defense

- Active in binding (fibers, yeast walls, bacterial walls, etc..)

- Active in deactivation/degradation (Protozoa, Bacteria, etc…)

Mycotoxin	Main product of rumen metabolism	Reduction of biological potency	Estimated carry-over rates
Aflatoxin B1	aflatoxicol	minor	n.d.[b] 0–12.4 $\mu g\,l^{-1}$[c]
	aflatoxin M_1[d]	minor	2.0–6.2%
Cyclopiazonic acid	unchanged	unchanged	n.d. 6.4–0.7 $\mu g\,l^{-1}$[e]
Fumonisin B1	unchanged	unchanged	0–0.05%
Ochratoxin A	ochratoxin-α	significant[f]	n.d.
T-2 toxin	various	significant	0.05–2%
DON (and related trichothecenes)	de-epoxy-DON (DOM)	significant	DON: 0.0001–0.0002 DOM: 0.0004–0.0024[g]
Zearalenone	α-zearalenol	none	0.06–0.08%[h]
Patulin[i]	unchanged	unchanged	n.d.
Ergovalin	unchanged	unchanged	n.d.
Lolitrem	unchanged	unchanged	n.d.

Fink-Gremmels, 2008

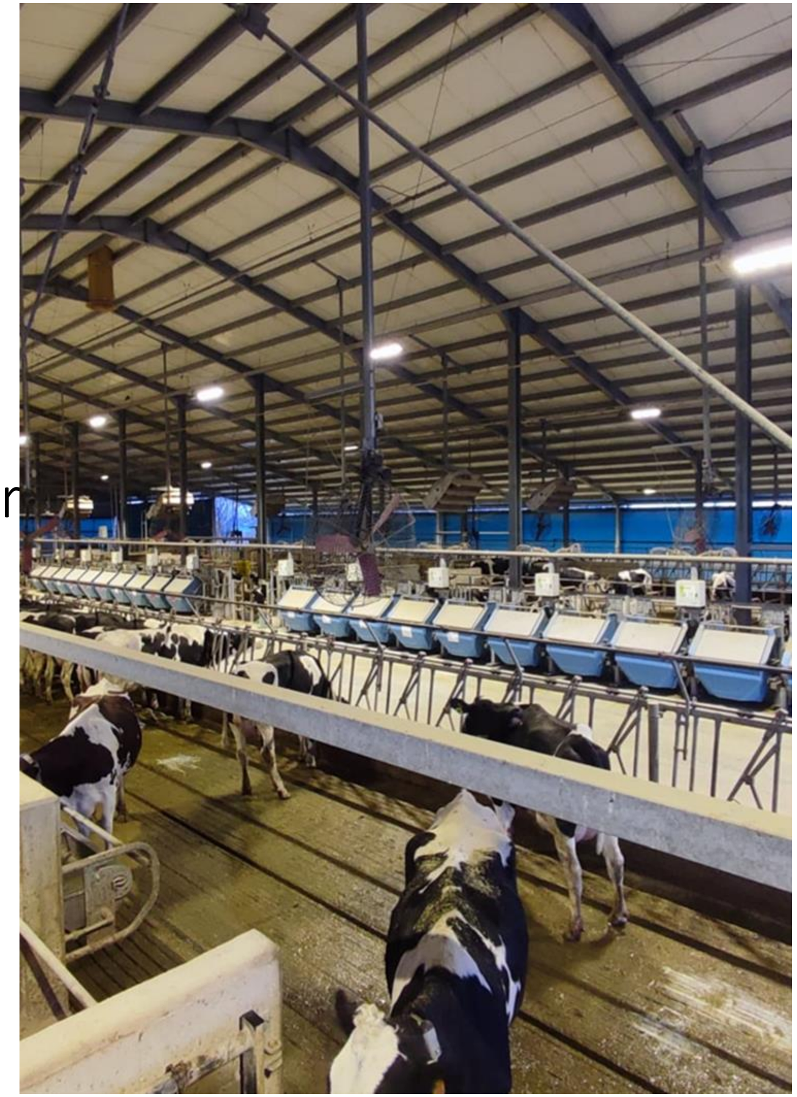
ZEA&DON trial In Cerzoo, UCSC experimental station

- 30 cows (21 multiparous e 9 first milking)
- 0 – 56 DIM
- From Winter 2022 to Spring 2023
- 3 experimental diets:

CTR = low contamination level

MXT = high contaminatio level

TRT = high contamination level + MDP (mycotoxin deactivating pr



✓ *Mil production and quality*

✓ *Immune-metabolic profile*

✓ *DMI*

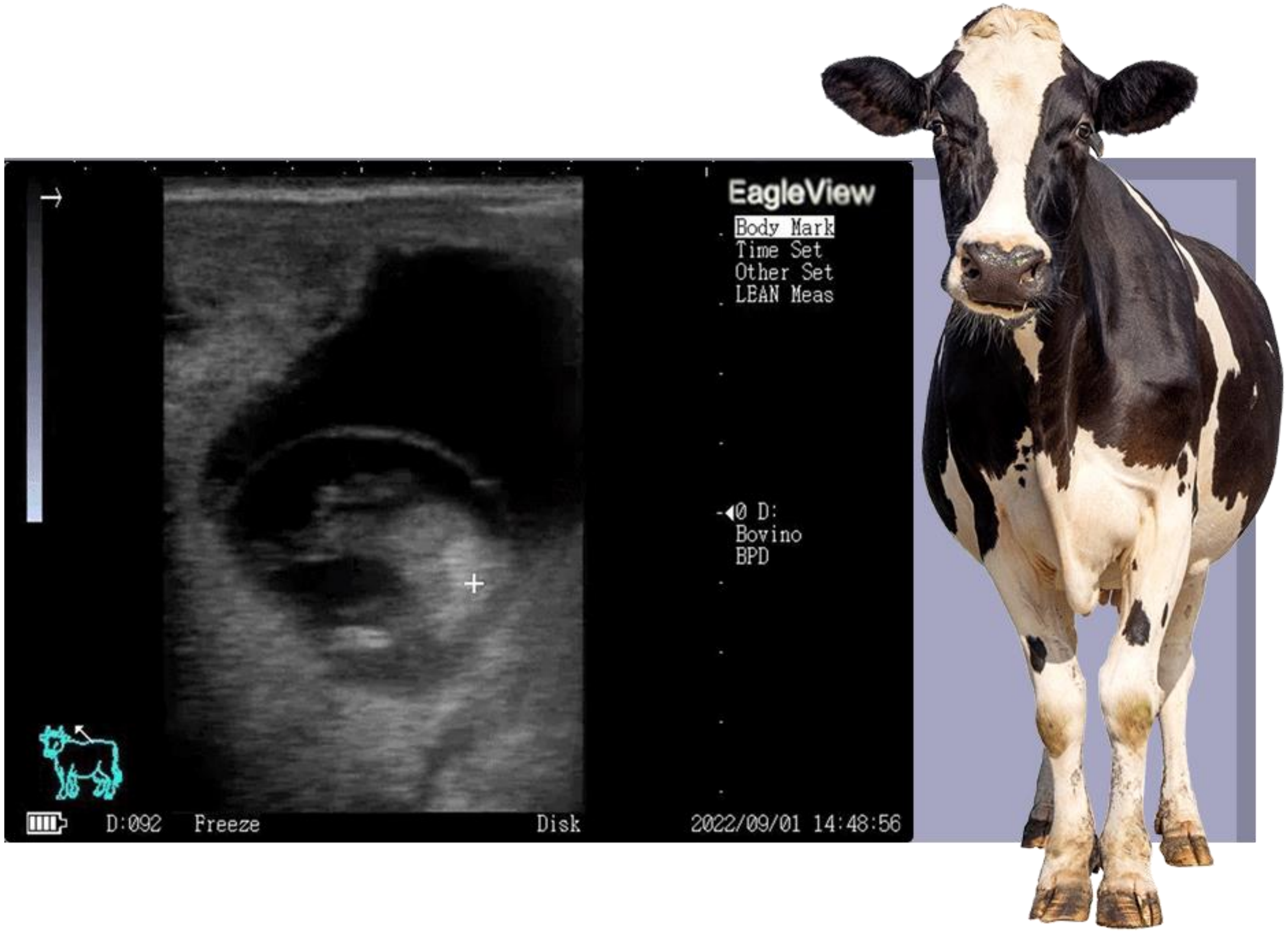
✓ *Rumination time*

✓ *Apparent nutrient digestibility*

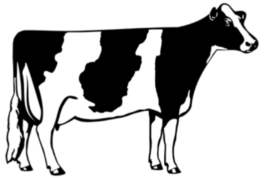
Monitoring Reproduction Performance

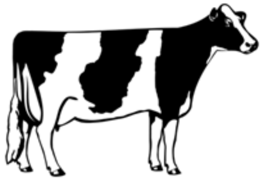
Items:

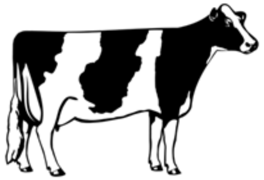
- Uterine evolution
- Ultrasonography weekly
- Number and dimension: corpus luteum, follicle, follicular cyst
- Heat detection
- Reproductive performances after experimental period
- Weekly progesterone



Experimental design







0°-6° — *Adaptation*

7°-56° — *Experimental trial*

>57° — *Out experimental trial*

Experimental TMR

Control TMR

	Diet		
	CTR	**MTX**	**MDP**
Fumonisin 1 (FB1)	578.79	613.49	559.56
Fumonisin 2 (FB2)	313.60	338.06	282.88
Zearalenone (ZEA)	**55.42a**	**366.63b**	**319.72b**
Deoxynivalenol (DON)	**226.8a**	**1141.54b**	**1028.42b**

Natural heat detection

Double ovsynch

1° FA

WWP = 83 DIM

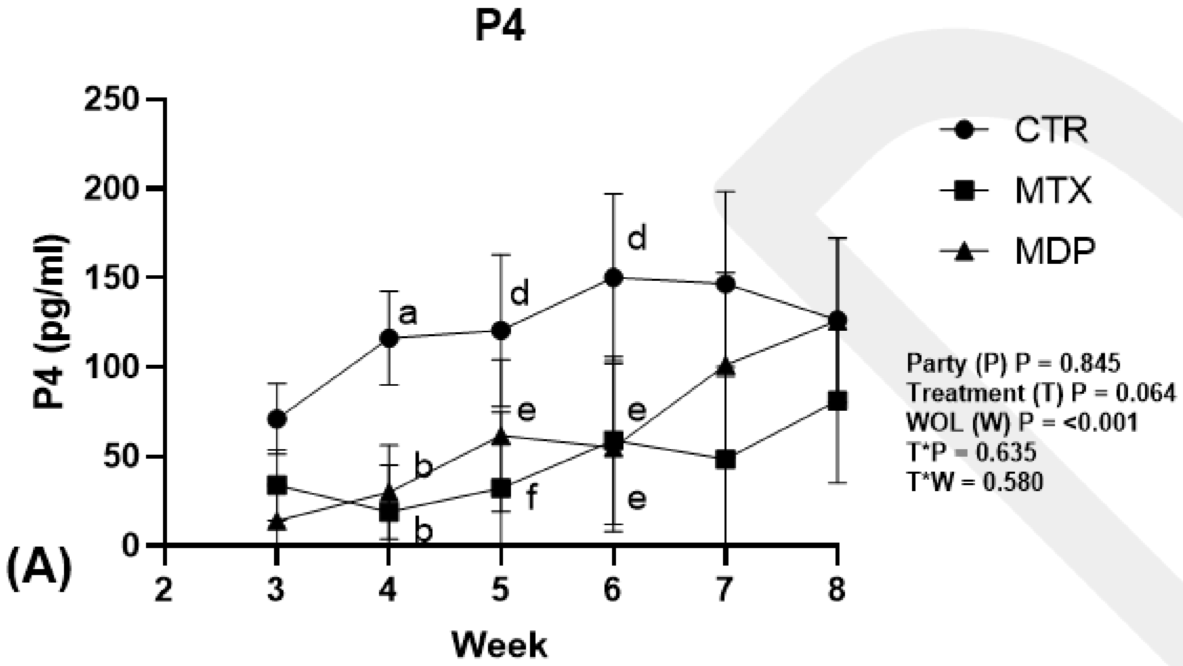
ZEN e milk

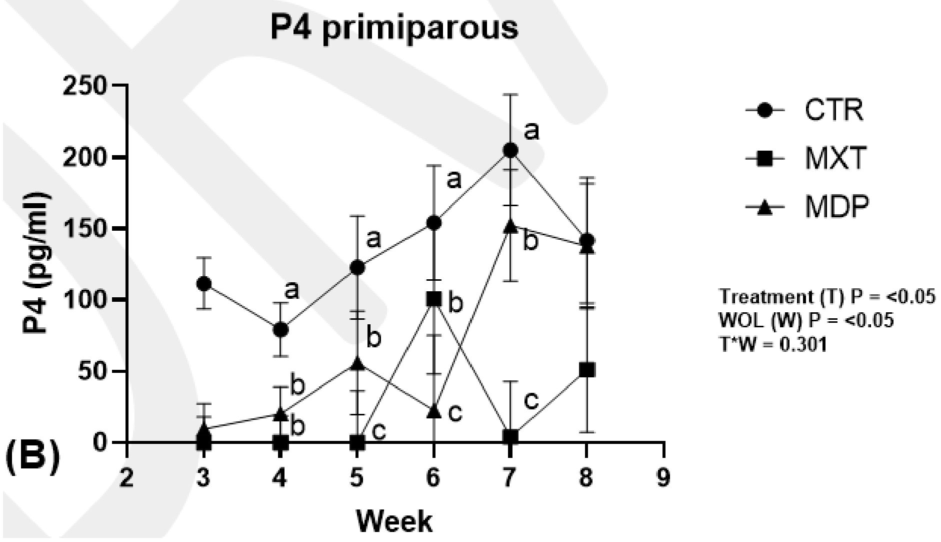
Items		Parity		Diet			Sem	P of the model				
		Primiparous	Multiparous	CTR	MTX	MDP		Parity (P)	Treatment (T)	WOL (W)	P * T	T*W
Milk yield	Primip+Multip	35.91	45.56	42.11	38.88	41.21	0.339	<0.0001	0.440	<0.05	0.421	0.283
(kg/d)	Primiparous			35.31	35.13	37.29	0.303		0.721	<0.05		0.980
	Multiparous			48.91	42.63	45.14	0.624		0.237	<0.05		0.795
FCM	Primip+Multip	34.90	43.24	40.60	37.05	39.56	0.797	<0.05	0.465	<0.05	0.289	0.971
(kg/d)	Primiparous			33.75	34.11	36.83	0.756		0.506	<0.05		0.338
	Multiparous			47.45	39.99	42.28	1.278		0.214	<0.05		0.271
ECM	Primip+Multip	34.55	42.66	40.33	36.48	39.00	0.672	<0.05	0.402	<0.0001	0.299	0.922
(kg/d)	Primiparous			33.69	33.50	36.45	0.573		0.497	<0.0001		0.869
	Multiparous			46.97	39.47	41.54	1.155		0.194	<0.0001		0.441
Feed efficiency	Primip+Multip	2.06	1.93	2.02	2.01	1.95	0.007	0.200	0.822	<0.0001	0.910	0.810
(dmnl^2)	Primiparous			2.11	2.07	1.99	0.012		0.697	<0.0001		0.741
	Multiparous			1.94	1.94	1.92	0.010		0.987	<0.0001		0.784
Fat	Primip+Multip	3.87	3.75	3.75	3.77	3.90	0.042	0.444	0.708	<0.0001	0.636	0.613
(wt/wt)	Primiparous			3.70	3.89	4.04	0.063		0.461	<0.0001		0.115
	Multiparous			3.78	3.67	3.78	0.055		0.879	<0.05		0.819
Protein	Primip+Multip	3.12	3.08	3.13	3.03	3.13	0.002	0.598	0.399	<0.0001	0.332	0.125
(wt/wt)	Primiparous			3.13^{b}	2.99^{c}	3.21^{a}	0.002		0.068	<0.0001		0.064
	Multiparous			3.13	3.06	3.04	0.003		0.732	<0.0001		0.591
Lactose	Primip+Multip	4.78	4.70	4.79	4.73	4.71	0.002	<0.05	0.160	<0.0001	<0.05	0.536
(wt/wt)	Primiparous			4.86^{a}	4.81^{a}	4.68^{b}	0.004		<0.05	<0.0001		0.073
	Multiparous			4.73	4.65	4.73	0.003		0.366	<0.0001		0.916
Urea	Primip+Multip	26.19	28.33	26.35^{b}	29.31^{a}	26.11^{b}	3.846	0.071	0.050	<0.05	0.510	0.557
(mg/100ml)	Primiparous			24.93	29.12	24.23	9.967		0.107	0.066		0.602
	Multiparous			27.81	29.43	27.79	3.296		0.546	<0.05		0.367
SCC	Primip+Multip	287.36	152.69	68.21	258.57	333.30	35048	0.308	0.200	0.413	<0.05	0.415
log_{10} (cells/ml x 1,000)	Primiparous			83.18	138.70	615.63	82259		0.134	0.228		0.353
	Multiparous			53.23	363.57	41.26	35752		0.135	0.672		0.758
Casein	Primip+Multip	2.38	2.36	2.40	2.31	2.39	0.002	0.772	0.405	<0.0001	0.388	0.462
(wt/wt)	Primiparous			2.39	2.28	2.45	0.001		0.124	<0.0001		0.128
	Multiparous			2.42	2.34	2.32	0.003		0.611	<0.0001		0.867

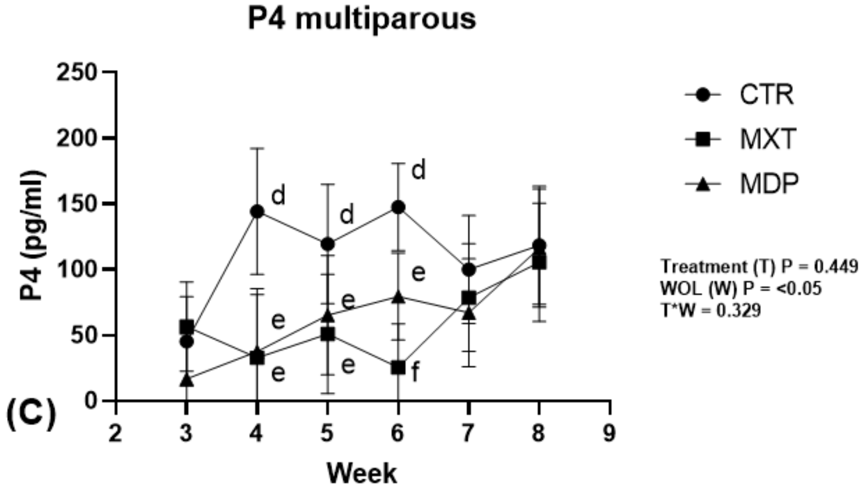
ZEN and immune metabolic profile

Items		Parity		Diet			Sem	P of the model				
		Primiparous	Multiparous	CTR	MTX	MDP		Parity (P)	Treatment (T)	WOL (W)	T * P	T*W
Packed cell volume	Primip+Multip	0.31	0.29	0.30	0.30	0.30	0.00005	<0.05	0.443	0.786	0.658	0.543
(vol/vol)	Primiparous			0.31	0.31	0.31	0.00007		0.888	0.230		0.097
	Multiparous			0.29	0.30	0.29	0.00006		0.327	0.815		0.089
Glucose	Primip+Multip	4.24	3.83	4.11	3.97	4.03	0.017	0.001	0.305	0.170	0.303	0.820
(mmol/L)	Primiparous			4.24	4.23	4.26	0.010		0.987	<0.05		0.769
	Multiparous			3.98[a]	3.70[b]	3.81	0.034		<0.05	0.888		0.833
Cholesterol	Primip+Multip	3.68	4.02	4.00	4.06	3.49	0.019	0.193	0.153	<0.0001	0.129	0.384
(mmol/L)	Primiparous			3.58	4.25	3.21	0.013		0.100	<0.0001		<0.05
	Multiparous			4.43	3.86	3.78	0.037		0.226	<0.0001		0.832
Urea	Primip+Multip	5.52	6.48	5.98	6.35[d]	5.67[e]	0.114	<0.05	0.159	<0.05	0.994	0.896
(mmol/L)	Primiparous			5.49	5.90	5.18	0.146		0.495	<0.05		0.555
	Multiparous			6.46	6.81	6.16	0.169		0.311	0.427		0.925
Total protein	Primip+Multip	77.61	81.20	79.18	80.59	78.45	1.095	<0.05	0.478	<0.0001	0.569	0.717
(g/L)	Primiparous			77.28	79.78	75.75	1.874		0.257	<0.0001		0.511
	Multiparous			81.07	81.39	81.14	1.511		0.990	<0.0001		0.557
Albumin	Primip+Multip	36.90	37.07	37.08	37.04	36.84	0.189	0.754	0.933	<0.05	0.767	0.274
(g/L)	Primiparous			36.83	37.23	36.64	0.367		0.864	<0.05		0.892
	Multiparous			37.33	36.84	37.05	0.247		0.830	<0.05		0.090
Globulin	Primip+Multip	40.70	44.13	42.10	43.55	41.60	0.892	<0.05	0.578	<0.0001	0.740	0.893
(g/L)	Primiparous			40.45	42.55	39.11	1.585		0.364	<0.0001		0.420
	Multiparous			43.74	44.55	44.10	1.119		0.955	<0.0001		0.262
GOT	Primip+Multip	91.90	91.50	95.08	90.14	89.87	42.495	0.936	0.618	<0.0001	0.090	0.326
(U/L)	Primiparous			87.84	92.69	95.16	132.84		0.787	<0.05		0.677
	Multiparous			102.42[a]	87.59[b]	84.59[b]	26.119		<0.05	<0.05		0.356
GGT	Primip+Multip	17.64	24.65	19.86	25.01	18.56	1.637	0.070	0.352	0.704	0.655	0.552
(U/L)	Primiparous			16.417	19.301	17.19	0.582		0.363	0.112		<0.05
	Multiparous			23.30	30.72	19.92	2.904		0.335	0.264		0.055

ZEN e progesterone

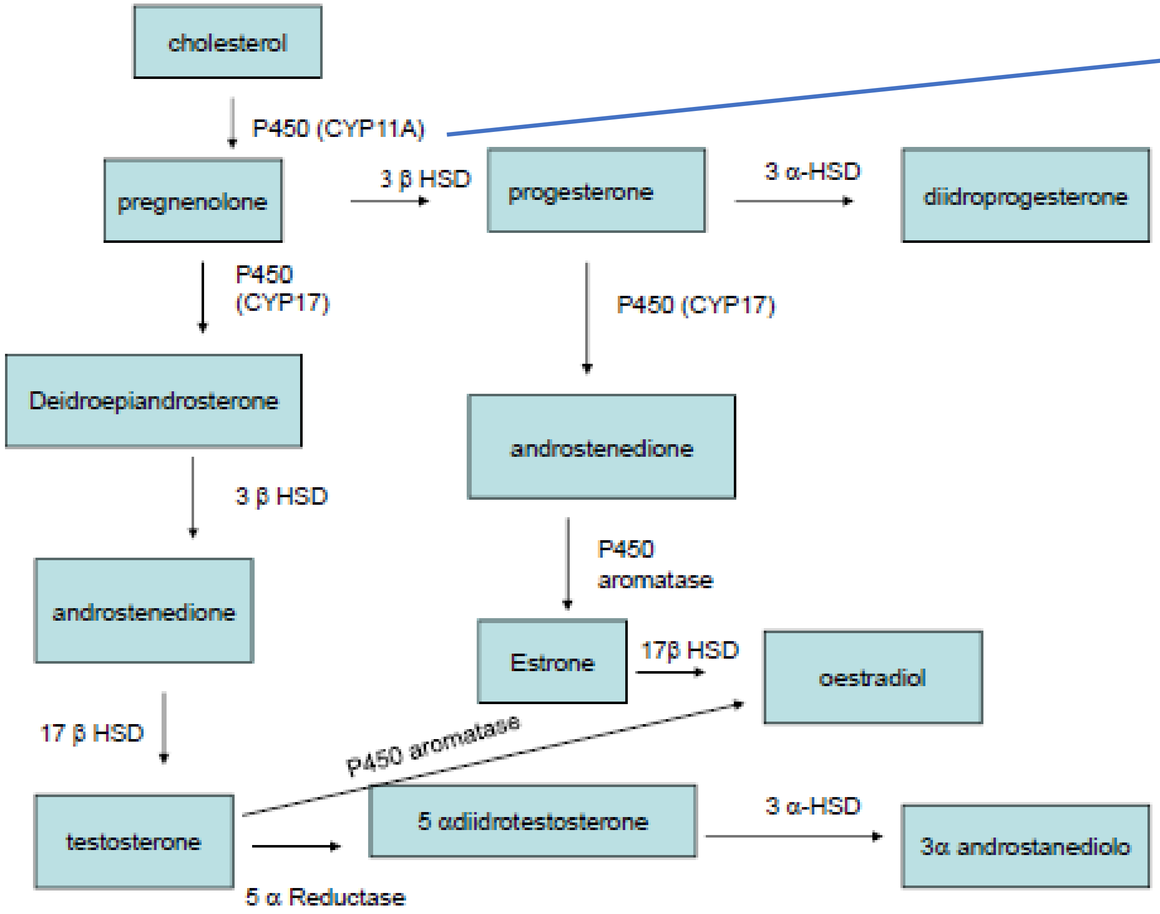




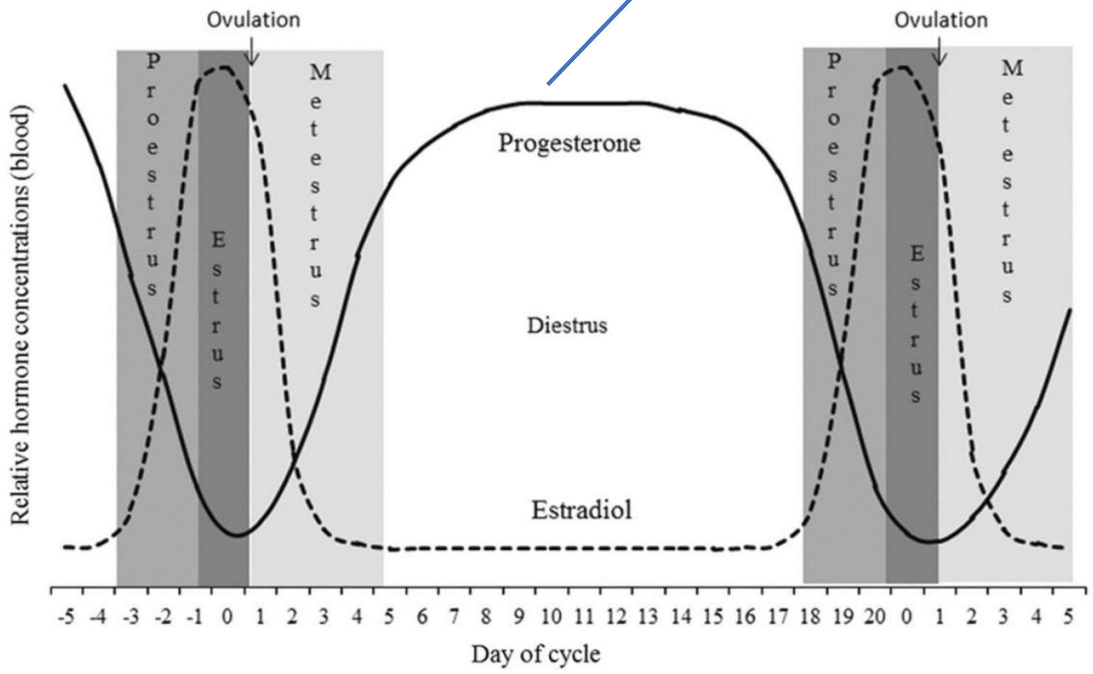


ZEN e P4….

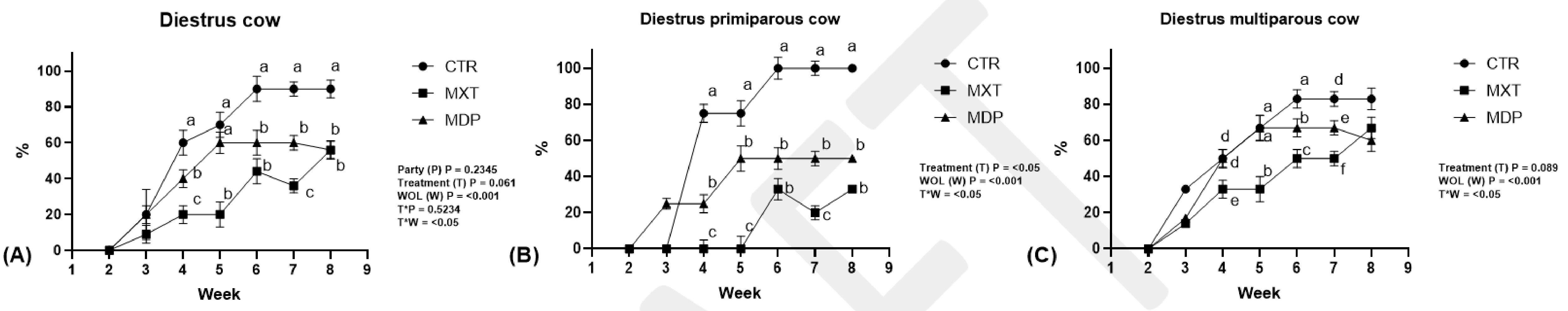
Figure 2. Biochemical pathway from cholesterol to steroid hormones.



ZEN is a susbtrate that modify the biochemical pathways of progesterone

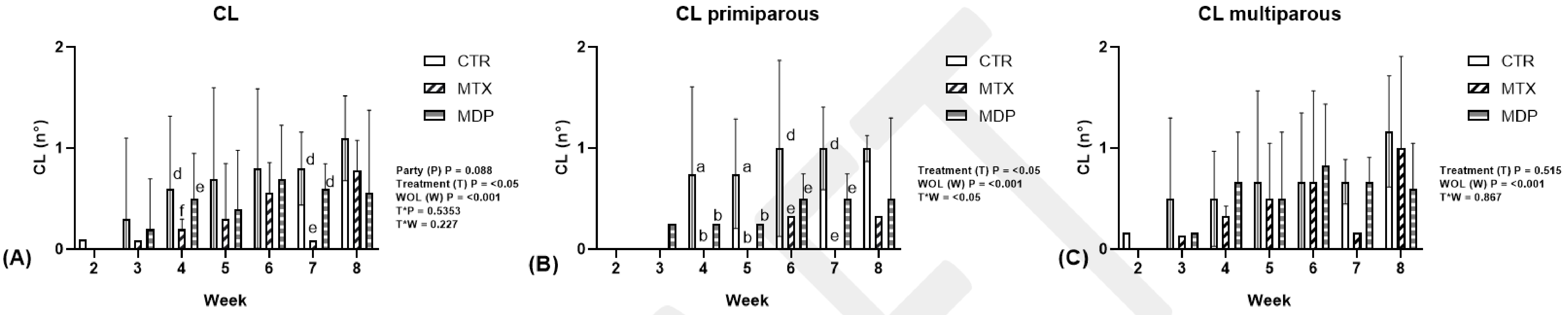


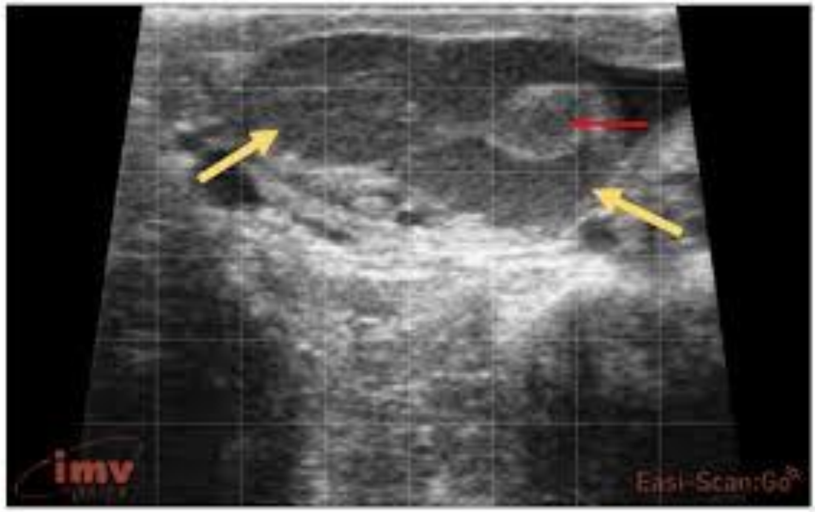
Cyclic and anovular cows

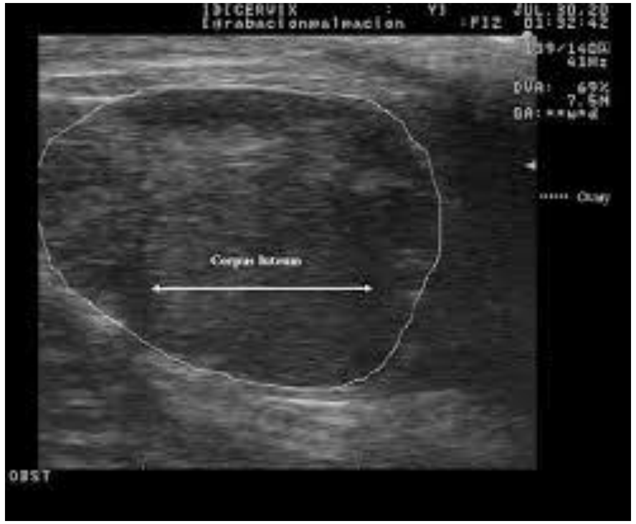


Big difference in number of cyclic cows in first 60 days. Mycotoxin contamination decrease % of cyclic cows by week and increase % of anovular cows

Corpus Luteum







Ovarian structure

Items		Parity		Diet			Sem	P of the model				
		Primiparous	Multiparous	CTR	MTX	MDP		Parity (P)	Treatment (T)	WOL (W)	T*P	T*W
Uterine Clearence (UC, scale 1-4)	Primip+Multip	1.27	1.52	1.34	1.42	1.43	0.046	0.096	0.878	<0.001	0.063	0.491
	Primiparous			1.14	1.25	1.43	0.151		0.184	<0.05		<0.05
	Multiparous			1.54	1.60	1.43	0.196		0.818	0.007		0.300
CL diameter, (cm)	Primip+Multip	2.12	2.09	2.05	2.41	2	0.088	0.995	0.903	<0.05	0.963	0.307
	Primiparous			2.16	2.65	1.02	0.152		0.722	0.181		0.539
	Multiparous			1.98	2.37	2.04	0.140		0.938	0.066		0.438
AFC, (n)	Primip+Multip	2.33	2.32	2.37	2.53	2.10	0.039	0.950	0.434	<0.05	0.684	0.483
	Primiparous			2.21	2.60	2.21	0.058		0.508	0.005		0.772
	Multiparous			2.52	2.45	1.99	0.054		0.466	<0.05		0.660
F1 diameter, (mm)	Primip+Multip	1.32	1.25	12.4	12.8	13.3	0.018	0.260	0.514	0.658	0.676	0.980
	Primiparous			13.2	12.9	13.3	0.032		0.962	0.129		0.319
	Multiparous			11.8	12.3	13.5	0.022		0.097	0.800		0.844
COF number, (n)	Primip+Multip	0.04	0.24	0.05	0.22	0.15	0.005	<0.05	0.409	<0.05	0.514	0.262
	Primiparous			0.04	0.08	0	0.002		0.510	0.169		0.654
	Multiparous			0.07	0.36	0.29	0.009		0.298	<0.05		0.293
COF diameter, (cm)	Primip+Multip	3.12	3.45	3.25^{b}	3.5^{a}	3.38^{b}	0.357	0.960	<0.05	0.365	0.555	0.224
	Primiparous			3	3.25				0.06	0.351		0.368
	Multiparous			3.33^{b}	3.54^{a}	3.38^{b}	0.415		<0.05	0.373		0.328

CL = corpus luteum; AFC = antral follicle counts; F1 diameter = mean diameter of the largest follicle; COF = mean number of follicular ovarian cyst; COF diameter = mean diameter dimension of follicular ovarian cyst.

Incidence of follicular cysts

Reproductive performance

Item	Parity	Treatment			Sem	P of the model	
		CTR	MTX	MDP		Parity	Treatment (T)
Days open, DO	Primip+Multip	105.12	105.00	110.90	42.71431	0.9256	0.9443
(n)	Primiparous	111.75	108.00	102.75	41.21421		0.9533
	Multiparous	98.50	102.00	116.33	48.43959		0.8262
Conception rate at first TAI, CR 1st TAI	Primip+Multip	50.00	62.50	50.00	52.64731	0.7896	0.8564
(%)	Primiparous	25.00	75.00	50.00	52.70463		0.4402
	Multiparous	75.00	50.00	50.00	54.35573		0.7451
CR in the entire lactation	Primip+Multip	0.76	0.75	0.70	0.337966	0.9123	0.9285
(%)	Primiparous	0.71	0.82	0.71	0.354371		0.8925
	Multiparous	0.82	0.69	0.70	0.362284		0.8610
Services per conception, S/C	Primip+Multip	2.00	1.78	1.90	1.1678	0.8653	0.6863
(n°/pregnancy)	Primiparous	2.00	1.60	2.00	1.045		0.5245
	Multiparous	2.00	2.00	1.86	1,213		0.7478
Anovular cows,	Primip+Multip	0.20	0.40	0.30	0.475317	0.0879	0.6469
(%)	Primiparous	0.00[C]	0.75[A]	0.50[B]	0.440959		0.099
	Multiparous	0.33	0.17	0.17	0.447214		0.7613
Calving to first estrus behavior interval, 1HD,	Primip+Multip	24.40[A]	45.75[B]	32.75[C]	11.91931	0.0967	0.0673
(d)	Primiparous	38.00	45.00	27.00	12.72792		0.6390
	Multiparous	21.00[A]	46.00[B]	38.50[B]	12.20997		0.0846
Calving to first ovulation interval 1OV,	Primip+Multip	24	29.5	25.14	9.12	0.576	0.527
(d)	Primiparous	24.5	33	26	7.01		0.597
	Multiparous	23.5	28.8	24.8	10.80		0.743

Mycotoxins and Ruminants

Diet of ruminants are much more diversified

- Concentrate
- Protein rich feed
- **Fibrous** and no-fibours **By-products**
- **Silage (corn silage, sorghum silage, small grain silage, legume silage, mix gras-legume silage, haylage, etc.)**
- **Hay (alfalfa hay, ryegrass hay, grass hay, etc.)**
- **Meadows and pastures**

Feeds	Possible mycotoxin contamination
Concentrate	aflatoxins, fumonisins, ZEA, DON, other trichothecenes, ergot alkaloids, etc.
Silage	patulin, mycophenolic acid, roquefortines, fumitremorgens, cerruculogen, monacolines, etc.
Hay	Alternaria toxins, Cyclopiazonic acid, DON, other trichothecenes, etc.

Fink-Gremmels, 2008

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

Gallo et al. Toxins 2021, 13, 232.

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).

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

Gallo et al. Toxins 2021, 13, 232.

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)

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

Gallo et al. Toxins 2021, 13, 232.

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

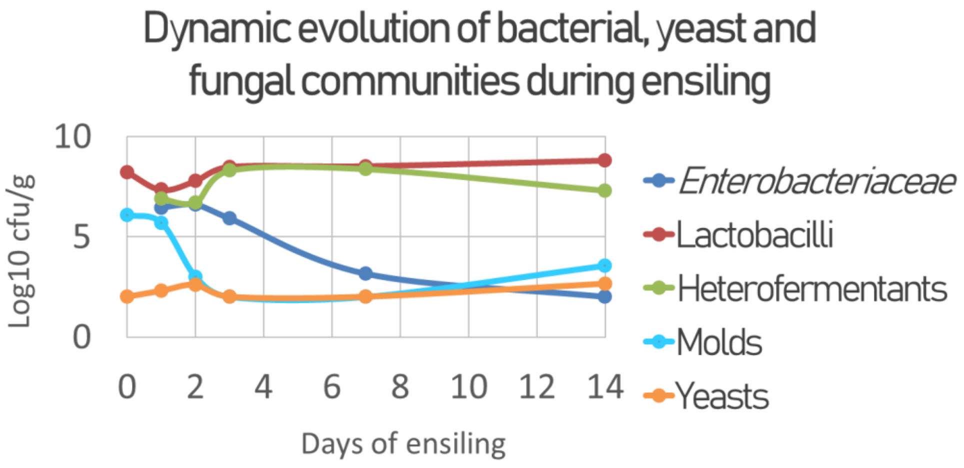
Phytoestrogens

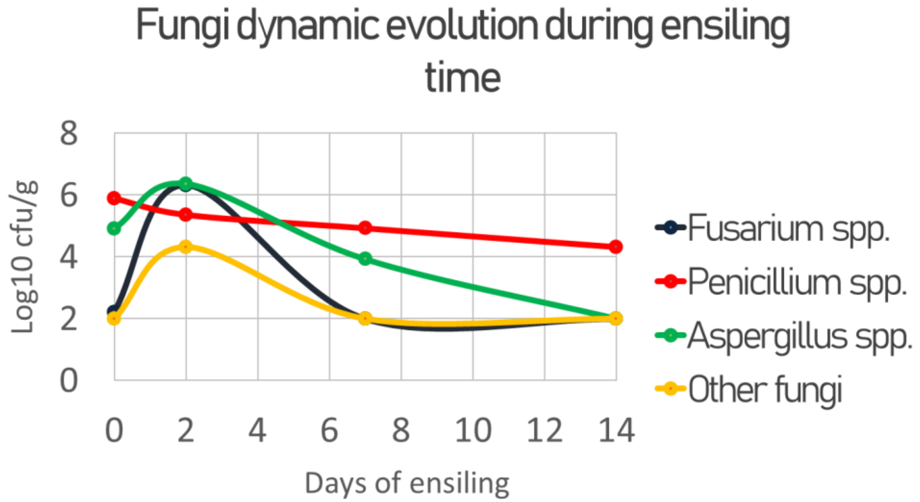
- Biochanin, Daidzein, Daidzin, Genistein, Genistin, Glycitein, Glycitin, Ononin, Coumestrol

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

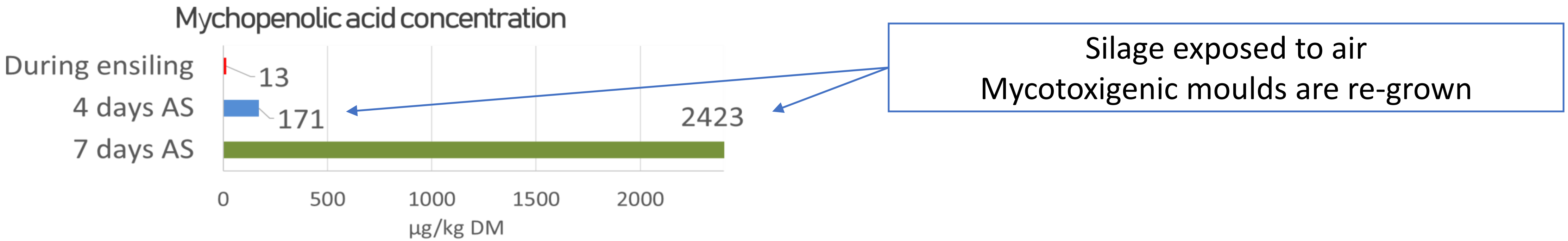
Gallo et al. MycoKey - Bari, 9 to 12 November 2021

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; Ogunade et al., 2018).





Unexpected → 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)



Relationship between contaminated corn silage and milk metabolomic profile

Rocchetti, G.; Ghilardelli, F.; Bonini, P.; Lucini, L.; Masoero, F.; Gallo, A. 2021. Metabolites, 11, 475.

45 milk samples were classified into five clusters based on the mycotoxin contamination profile of corn silage linked to the corresponding milk samples.

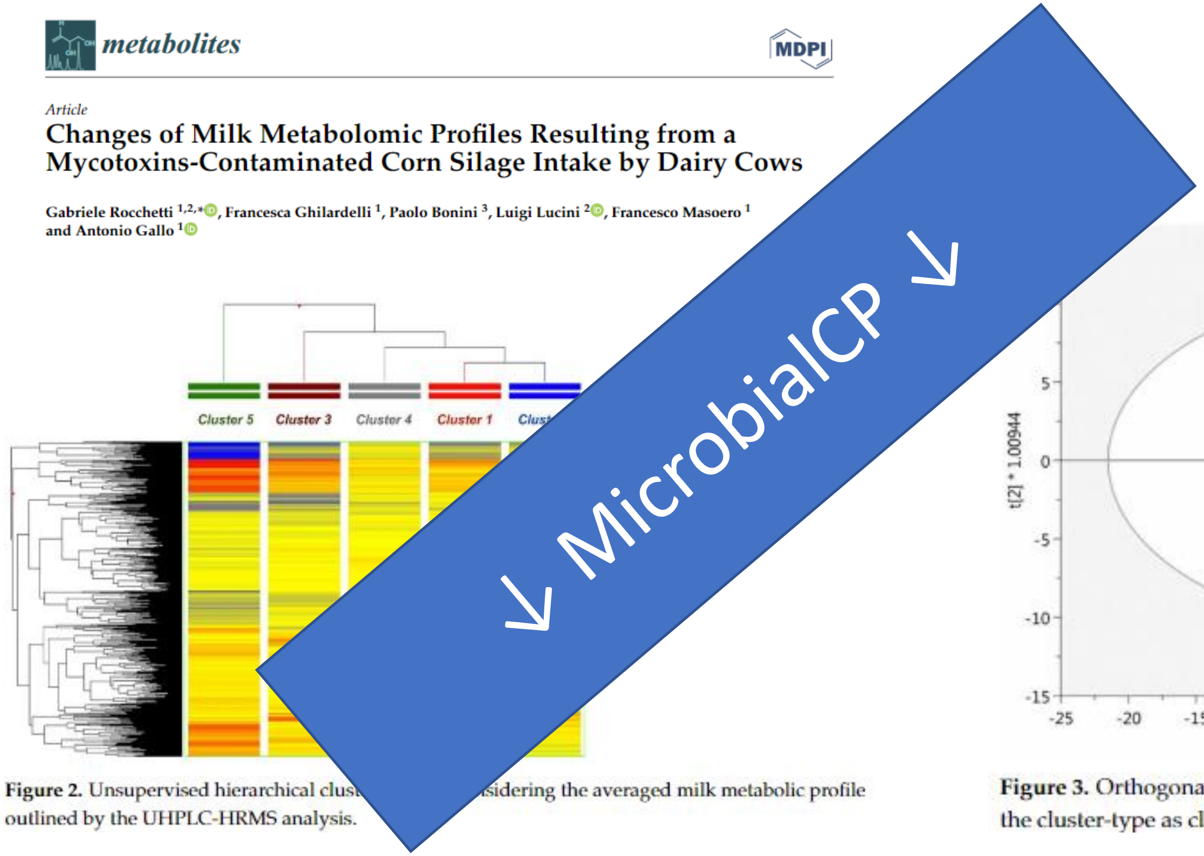


Figure 2. Unsupervised hierarchical clus… …sidering the averaged milk metabolic profile outlined by the UHPLC-HRMS analysis.

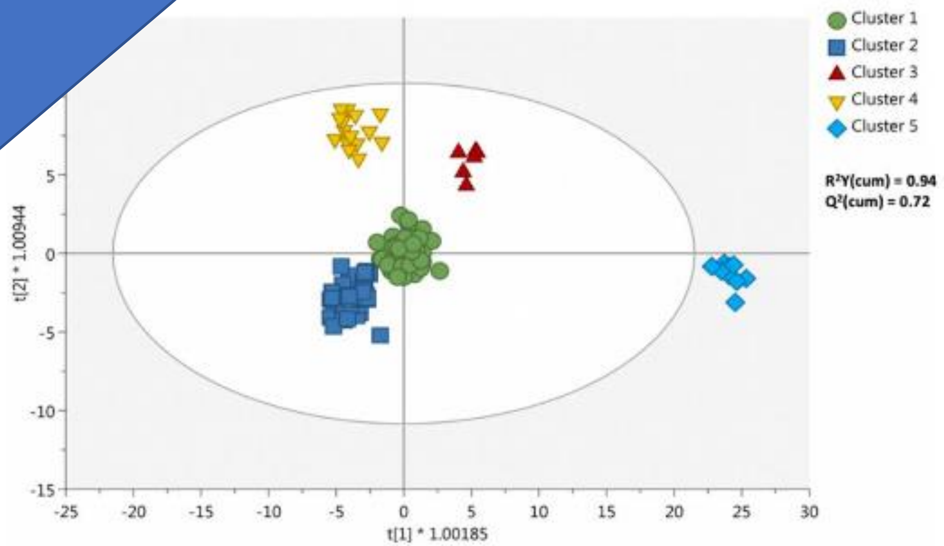


Figure 3. Orthogonal projection to latent structures (OPLS) discriminant analysis (DA) considering the cluster-type as class discrimination parameter.

ANALYSIS OF THE **METABOLOMIC PROFILE OF THE MILK** SHOWED CORRELATIONS BETWEEN THE **QUALITY OF CONTAMINATED CORN SILAGE,** THAT WAS PART OF THE RATION, AND THE COMPOSITION OF THE MILK, WITH THE PRESENCE OF METABOLITES SUCH AS AMINO ACIDS AND PEPTIDES, FOLLOWED BY **PURINE, PYRIMIDINES** AND STEROID CONJUGATES.

Take of Message

- **Mycotoxins** are deeply studied in animals, but for ruminants we have still few data for obtaining final statements
  - Eg: effect on feeding behavior, feed digestibility, intestinal health status or milk quality parameters

- A lot of regulated and emerging ***Mycotoxins*** can contaminated feeds, also in silage and haylage, being characterized by complex microflora, different among ensiling phases (next presentation)

- What about By-Products or Co-Products used in Animal diets (#SafetyOfBy-products - SOB)???

- Common protocols for testing effect of mycotoxins in ruminants should be adopted

- People involve in this topic should work together to increase level of knowledge

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